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HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ROBERT LARSSON

BAND XXIX HÄFT. 1—2

LUND 1943, BERLINGSKA BOKTRYCKERIET

HEREDITAS,

a periodical devoted to the publication of original research in heredity, is published by the MENDELIAN SOCIETY at Lund, Sweden. All contributions are written in English, German or French, illustrated, when necessary, with adequate text figures and plates. Each volume contains about 400 pages, issued in four numbers.

Subscriptions should be sent to the Editor. Price *Twenty-five (25) Swedish Kronor per volume.* Post free. (The price of Vols. I—X is 10 Swedish Kronor per volume, of Vols. XI—XXIII, 15 Sw. Kr., and of Vols. XXIV—XXV, 20 Sw. Kr.)

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CYTOLOGICAL STUDIES ON THE FAMILY CYPRINODONTIDAE

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I. PREVIOUS INVESTIGATIONS.

1. XIPHOPHORINI.

CYTOLOGICAL examinations of the tribe Xiphophorini have been carried out by FRIEDMAN and GORDON (1934) and by RALSTON (1934). FRIEDMAN and GORDON treat of the following species: *Xiphophorus helleri* and *montezumae*, *Platypoecilus maculatus*, *couchianus*, *variatus* and *xiphidium*, and the hybrids *Pl. couchianus* × *maculatus*, *xiphidium* × *maculatus*, *variatus* × *maculatus* and *X. helleri* × *Pl. maculatus*. The research is based entirely on testes and an account of the number of chromosomes at the first spermatocyte division is given. At the spermatogonial divisions the chromosomes are said to be small and uniform. At meiosis the above-named investigators find 24 bivalents in all animals except *Pl. variatus* and its hybrids. *Pl. variatus*, on the contrary, has 24 bivalents and one univalent. In the hybrids *Pl. variatus* × *maculatus* either the same condition is found as in *Pl. variatus* or 23 bivalents and one trivalent, which, according to FRIEDMAN and GORDON, points to the fact that *Pl. variatus* resulted through fragmentation of one of the chromosomes of *maculatus*. As regards these species and hybrids the above-mentioned authors have found no cytological evidence of heterochromosomes. The cytological conditions agree with KOSSWIG's (1931, 1935 a and b, 1936, 1937) theories of homologies of the sex chromosomes within this tribe.

RALSTON (1934) has cytologically examined the following species within the tribe Xiphophorini: *Pl. maculatus*, *couchiana*, »two species of *Xiphophorus*» and »the hybrids *Xiphophorus* × *Platypoecilus*». RALSTON does not, however, specify which of the *Xiphophorus* species he examined. This omission unfortunately reduces to a high degree the value of his work.

Contrary to FRIEDMAN and GORDON, RALSTON has examined the whole spermatogenesis. At meiosis RALSTON finds 24 bivalents in all species examined by him. He also states that the largest chromosome

(»possibly the sex chromosome») lags behind the others at early metaphase and remains at some distance from the metaphase plate »attached to it by a special spindle fibre». In some cases it »divides» before the others and goes first towards the poles at anaphase. The second division is normal.

Hence RALSTON draws the conclusion that in the species examined the males should be »of the ZZ-type or of an XY-type where X and Y are morphologically alike». — »Since in other animals the X and Y are seldom morphologically identical, one is led to the assumption that the sex chromosomes are of the ZZ-type». This corresponds only regarding *Pl. maculatus* with the results obtained by KOSSWIG and others on the basis of genetical work. That *X. helleri* is not of this type has been many times proved (REGNIER, 1938 *et lit. ibidem cit.*); concerning the other species there is no genetical proof for one view or the other. RALSTON's hybrids where the parental species are not exactly stated evade all judgement. If the commonest and easiest produced *X.* × *Pl.* hybrid, *X. helleri* × *Pl. maculatus*, is concerned, RALSTON's idea obviously stands in opposition to KOSSWIG's, but for reasons which I will set forth later, the latter's appear to be the better founded.

From these investigations and the genetical works of BELLAMY (1928), FRASER and GORDON (1928), GORDON (1937) and KOSSWIG (1931, 1935 a and b, 1936, 1937) it is evident that the tribe is cytologically very unitary; hybrids are easy to produce and the chromosome pairing at meiosis is complete in all hybrids cytologically examined.

On the other hand, the differences in the sex determinating mechanism appear to be great. Both phenotypical and genotypical sex determination occur within the tribe, and of the latter the *Drosophila* as well as the *Abraxas* type is found. This is interpreted by KOSSWIG, WITSCHI and others to be owing to the fact that the heterochromosomes within this tribe, possibly within the entire family, are in the first stages of their evolution and only slightly developed. The apodeitic cytological proofs of this are missing as yet.

2. THE LIMIA SPECIES.

The conditions in the species of the genus *Limia*, examined by BREIDER (1935) are very like those of Xiphophorini. All BREIDER's investigations are purely genetical. Also in this genus both genotypical and phenotypical sex determination seem to occur. No cytological

investigation of any of the *Limia* species has to my knowledge appeared.

3. LEBISTES.

A comprehensive survey, further, has been made of the genetics and cytology of *Lebistes reticulatus*. Above all by WINGE's pioneer works (1922, 1923, 1927, 1930) the inheritance of the colour genes of *Lebistes* is made clear. Interesting experiments have also been made by WINGE concerning the sex determination, which appears to be according to the *Drosophila* scheme.

Some cytological works on *Lebistes* have also appeared. WINGE gives (1922 a) a brief survey of spermatogonial, oogonial and maturation divisions. As to the mitosis he mentions that the number of chromosomes is almost impossible to determine. At meiosis the number is 23 in both first and second divisions as well as at the diakinesis of the oocyte.

VAUPEL (1929) describes the entire spermatogenesis completely and well. He treats the mitosis rather cursorily, showing interest mainly for the mitochondriae, but indicates that the metaphase plate contains »approximately forty-six chromosomes as established by WINGE». At meiosis one chromosome pair appears, according to VAUPEL, to separate before the others at anaphase and therefore shows »some evidence to be the sex chromosomes».

The third and latest cytological examination of *Lebistes* has been carried out by IRIKI (1932). He only gives the number of chromosomes $n = 23$.

4. OTHER SPECIES.

Other species and genera within this family are still more incompletely known. GEISER (1924) has applied himself to *Gambusia holbrooki*. No noteworthy cytological observations are set forth here except that the number of chromosomes appears to be $n = 18$. GEISER observed irregularities in the divisions, possibly explained by the presence of heterochromosomes.

MEYER (1938) has made a count of the chromosomes of *Mollienisia sphaenops* and *M. latipinna* and found $2n = 36$, $n = 18$ in both species. The hybrid between them »carries the diploid number of chromosomes».

Thus one may state that many species of this family are quite well known genetically, while the cytological conditions have been

examined to a very small extent. The species in question, especially regarding sex determination, have a unique position as the only case hitherto known among Vertebrata where all known types of sex determination appear in so closely related forms that species of the same genus capable of hybridizing show different types of sex determination. If this is, as KOSSWIG, BREIDER and WITSCHI (1934) think, a primitive characteristic or a secondary non-balance can hardly as yet be decided. To solve this and other problems connected with it, more accurate cytological examinations are required. This work is intended to be a step in this direction.

II. OWN RESULTS.

1. MATERIALS AND METHODS.

The main material for this investigation consists of testes of mature individuals. As fixative I have used BOUIN's, FRIEDMAN and GORDON's modification of NAVASHIN's, and FLEMING's strong solution. BOUIN's has given excellent results on *Lebistes* and *Xiphophorus* but fixes especially the *Mollienisia* species, *Limia* and *Platypoecilus* poorly, while NAVASHIN's has always, except in *Platypoecilus variatus*, afforded excellent results. The imbedding has been made in paraffin over methyl benzoate, and the slices cut 10 microns in thickness. The stain used was EHRLICH's and HEIDENHAIN's haematoxyline. I have also used smear preparations according to the permanent acetocarmine method. These give clear and good pictures. Of embryos I have examined partly section series of 7—15 mm individuals, partly permanent acetocarmine preparations of 3 mm stages.

All figures are drawn in a magnification of 5000 × with the aid of a camera lucida and reduced to about $^1/_2$ for reproduction. The optics used was a Zeiss' binocular microscope with a Leitz' $^1/_{16}$ obj. (apert. 1,32; 112 ×) and Zeiss' comp. eyepieces 20 ×, giving a magnification of 3360 ×. All drawn figures are polar views of metaphase plates. Fixation (except Fig. 7) FRIEDMAN and GORDON's modification of NAVASHIN's fluid. Stain HEIDENHAIN's haematoxyline.

2. MITOSIS.

A. NUCLEOLI.

The primordial germ cells differ in the Cyprinodonts examined by me in no way from what is normal. They always contain one or more nucleoli. The number of nucleoli in various cells is within certain

limits constant in every species but varies sometimes from cell to cell in the same individual. I have found the following numbers in the different species:

Species	Number of nucleoli in Embr. soma	Prim. germ cells	Early spg.	Late spg.	Previous literature
Xiphophorus helleri	1—2	1	1	2	1. ESSENBERG (1923) 1. RALSTON (1934)
Platypoecilus maculatus ...	1—2	1—2	1—2	1—2	1. RALSTON (1934)
Pl. variatus	—	1	1—3	4	
X. helleri × *Pl. maculatus*	—	1	1	2	1. RALSTON (1934)
Mollienisia sphaenops.....	—	1	1	1—2	
M. sph. var. *melanistica* ...	—	1	1	1—2	
M. velifera	—	1	1	2	
Limia caudofasciata var. *tricolor*	—	1	1	1—3	
L. vittata	—	1	1	1—3	
Lebistes reticulatus.....	1—4	2	1—2	1—2	1. DILDINE (1936)
Phallichtys pittieri	—	1	1	3	
Heterandria formosa.....	—	—	1	2	
Glaridichtys falcatus	—	1	1	1	
Rivulus santensis (egg-laying species)	—	—	2	3	

In those species where the number varies, it appears to increase during the course of the spermatogonial divisions. The amount of nucleolar substance appears to be rather constant, but larger nucleoli may be substituted by several smaller. Possibly these smaller nucleoli, however, are not nucleoli in the real sense but rather only fragments of the larger primary body or bodies. This is more especially the case in *Limia caudofasciata* var. *tricolor*. As against this, species are found with very constant numbers, as *Platypoecilus maculatus*.

Where previous writers have observed the nucleoli, they also seem to have perceived their varying number. In *Lebistes* VAUPEL (1929) observed »usually one nucleolus in the spermatogonia», while DILDINE (1936) found »one single prominent plastosome» in primordial germ cells. In primordial germ cells in my material I have invariably found two nucleoli, one large and one small (Fig. 1).

ESSENBERG (1923) found a single nucleolus in primordial germ cells of *Xiphophorus*. The same thing is reported by RALSTON. In *Platypoecilus* RALSTON also found one nucleolus, while my material has two.

The reason for varying numbers of nucleoli within even the same

organism may lie, according to McCLINTOCK (DARLINGTON, 1937), in a competition of the accessible nucleolar material between a number of nucleolus organizers in the chromosomes. Should on the basis of this theory conclusions be drawn concerning the material at hand, it seems to me as if the dominance (using the word in the literal and not the genetical sense) of the stronger organizers were more decided in the primordial germ cells and the earlier spermatogonia than in the later

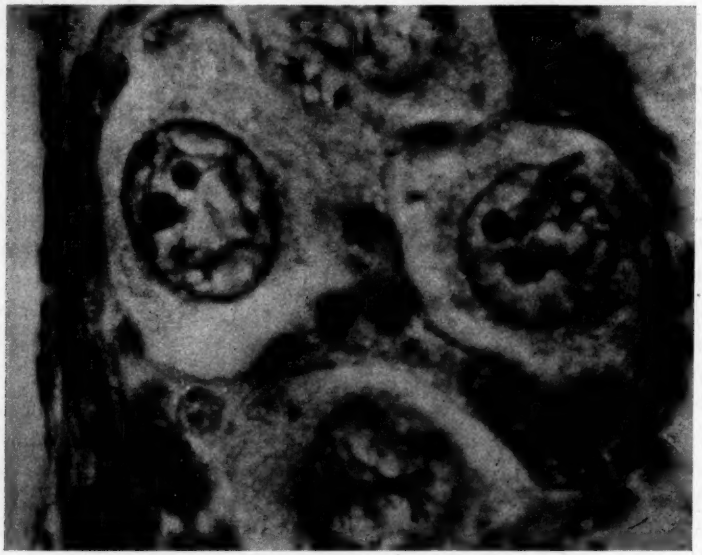

Fig. 1. Primordial germ cells of *Lebistes*. — 1500 ×.

and in embryonic somatic cells, but in the material in question fragmentation also may play a great rôle.

The mitotic divisions appear to follow the normal scheme. Any continuous spireme such as RALSTON describes in Xiphophorini does not naturally exist. Neither can I discover the polarization in the prophase of mitosis which RALSTON records as distinguishing the *Xiphophorus* species from *Platypoecilus*.

B. SOMATIC PAIRING.

When the chromosomes are orientated in the metaphase plate a peculiarity arises which appears to be characteristic of at least all Teleosts, possibly even of most Vertebrata. I allude to the somatic

pairing, i. e. that the chromosomes which belong to the same pair are lying near each other in the metaphase plate. How the somatic pairing is going on during the other phases I cannot determine owing to the small size of the cells, but it seems as if it continues, and might be explained by SVÄRDSON's (1941) theory.

Somatic pairing in Teleosts has been described before (SVÄRDSON and WICKBOM, 1939; SVÄRDSON, 1941), but other investigators do not appear to have observed it in fishes, though it may be traced from their figures.

Any connection between somatic pairing and abnormal meiosis such as is described in *Sciara* and *Drosophila pseudoobscura* (DARLINGTON, 1937) I cannot find in Cyprinodontidae. Chiasmata are found in all chromosomes of the males. WINGE (1922) has found the same in the female of *Lebistes*. The genetical observations also show that crossing over takes place at least between the sex chromosomes of *Platypoecilus* and *Lebistes*.

In the species and hybrids examined I have been able to establish the following chromosome numbers:

Species	2n =	n =	Previous literature	2n =	n =	Figs.
Xiphophorus helleri HECKEL	48	24	FRIEDMAN and GORDON (1934)	—	24	—
			RALSTON (1934)	48	24	4 and 14
Platypoecilus maculatus GUENTHER	48	24	FRIEDMAN and GORDON (1934)	—	24	—
			RALSTON (1934)	48	24	3 and 15
Pl. variatus MEEK	—	25	FRIEDMAN and GORDON (1934)	—	25[1]	16
X. helleri × *Pl. maculatus*	48	24	FRIEDMAN and GORDON (1934)	—	24	2, 17 and 18
			RALSTON (1934)	48	24	—
Lebistes reticulatus PETERS	46	23	WINGE (1922)	46	23	7, 19 and 20
			VAUPEL (1929)	46	23	—
			IRIKI (1932)	—	23	—
Phallichtys pittieri MEEK	46	23		—	—	6, 21 and 22
Phalloceros caudimaculatus var. *reticulatus* KÖHLER	46	23		—	—	32 and 33

[1] 24 bivalents and one univalent.

Species	2n=	n=	Previous literature	2n=	n=	Figs.
Limia vittata POEY	46	23		—	—	5, 23 and 24
L. caudofasciata var. *tricolor* STOYE (*L. dominicensis* REGAN).....	46	23[1]		—	—	25
Mollienisia sphaenops CUV. et VAL.	46	23	MEYER (1938)	36	18	11, 27 and 28
M. sphaenops var. *melanistica* WICKBOM	38	19[2]		—	—	9 and 29
M. sph. var. *melanistica* × *M. sph.*	42	21[3]		—	—	10 and 36
M. velifera REGAN	23	46		—	—	8 and 30
M. sph. × *M. velifera* ...	23	46		—	—	13 and 31
Heterandria formosa GIRARD	23	46		—	—	12 and 34
Rivulus santensis[4] KÖHLER	24	48		—	—	35

The diploid numbers correspond with the numbers other research-workers have given in the cases where the species have been the subject of examination, with the exception of *Mollienisia sphaenops*. I cannot give any explanation for this digression between the number MEYER found and mine. In my material the chromosome number at mitosis corresponds with that at the first and second meiotic division. MEYER states the same of the first division, but he does not mention the second.

MEYER's examination is partly made on individuals caught in nature, partly on aquarium tribes. As he reports that the former are fixed in formol and the latter in BOUIN's etc. fluids, one may assume that the cytological examination was carried out on aquarium tribes. My material is also aquarium animals. Examination of a number of individuals according to REGAN's (1913) key disclosed no digression from the data which are mentioned for *M. sphaenops*. As a rule they correspond very well with the figure MEYER publishes for his material.

As, however, I have been able to examine a large number of metaphase plates from spermatogonial, first and second meiotic divisions of this species, and collaborators have been kind enough to

[1] In 6 % of all cells 22 bivalents and 2 univalents.

[2] In 66 % of all cells. In 33 % numbers varying between 18 bivalents and 2 univalents and 14 bivalents and 10 univalents are found.

[3] 19 bivalents and 4 univalents.

[4] Egg-laying species.

verify these, there is no doubt that this material really has the chromosome number 2n = 46, n = 23. The result corresponds also with my findings in the hybrid with the black variety of this species, which will be dealt with later, and in the hybrid with *M. velifera.*

If, therefore, MEYER's number is correct for individuals examined by him, the divergence appears to be ascribable to different materials.

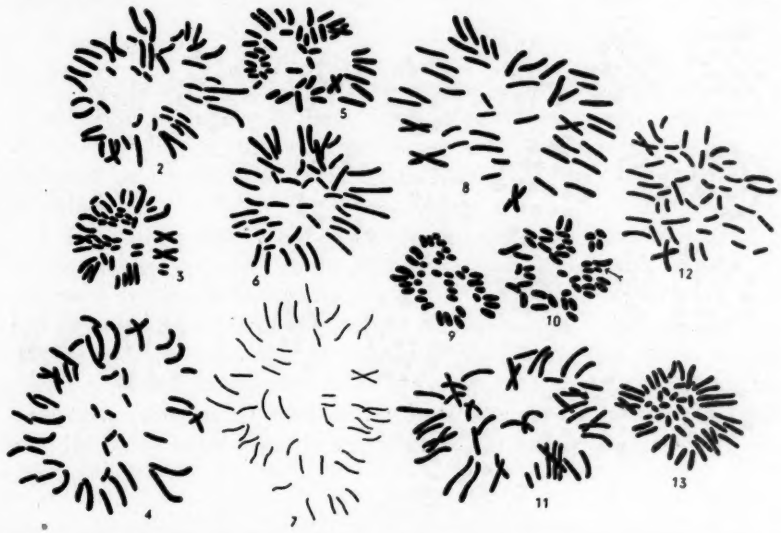

Fig. 2. Early spermatogonial generation of the hybrid *Xiphophorus helleri* × *Platypoecilus maculatus.* — Fig. 3. Late spg. of *Platypoecilus maculatus.* — Fig. 4. Early spg. of *Xiphophorus helleri.* — Fig. 5. Intermed. spg. of *Limia vittata.* — Fig. 6. Early spg. of *Phallichtys pittieri.* — Fig. 7. Early spg. of *Lebistes reticulatus.* Acetocarmine smear. — Fig. 8. Early spg. of *Mollienisia velifera.* — Fig. 9. Late spg. of *Mollienisia sphaenops.* var. *melanistica.* — Fig. 10. Late spg. of the hybrid *M. sphaenops* var. *melanistica* × *M. sphaenops.* The morphologically different members of one pair are marked by an arrow. — Fig. 11. Early spg. of *Mollienisia sphaenops.* — Fig. 12. Early spg. of *Heterandria formosa.* — Fig. 13. Late spg. of the hybrid *Mollienisia sphaenops* × *M. velifera.*

As I have further found the same number in *M. velifera* and in the very closely related genus *Limia,* I am convinced that the number given by me is correct for *M. sphaenops,* although certain tribes may diverge. It is also possible that on account of the somatic pairing often being especially intimate MEYER gave a wrong number for the mitosis. The main part of his examinations appears to have been carried out on this division, and the single figure he publishes is a mitotic metaphase

plate which shows an arrangement of the chromosomes that appears abnormal.

Concerning the morphology of the chromosomes of Cyprinodontidae very little is to be said. They are very short, smaller than 3 microns, and have terminal centromeres. I find it peculiar that in spite of every species possessing one or more nucleoli I have not been

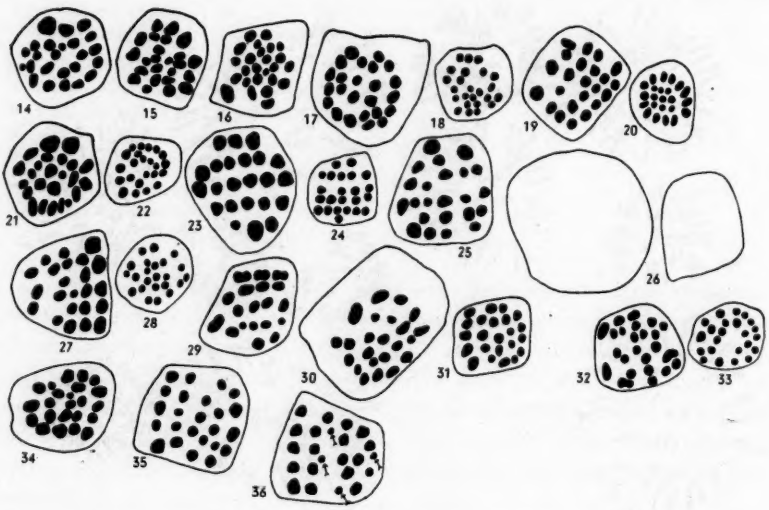

Fig. 14. M I of *Xiphophorus helleri*. — Fig. 15. M I of *Platypoecilus maculatus*. — Fig. 16. M I of *Platypoecilus variatus*. — Fig. 17. M I of *Xiphophorus helleri* × *Platypoecilus maculatus*. — Fig. 18. M II of *Xiphophorus helleri* × *Platypoecilus maculatus*. — Fig. 19. M I of *Lebistes reticulatus*. — Fig. 20. M II of *Lebistes reticulatus*. — Fig. 21. M I of *Phallichtys pittieri*. — Fig. 22. M II of *Phallichtys pittieri*. — Fig. 23. M I of *Limia vittata*. — Fig. 24. M II of *Limia vittata*. — Fig. 25. M I of *Limia caudofasciata* var. *tricolor*. — Fig. 26. Outlines of a tetraploid (left) and a diploid (right) cell at diakinesis of *Xiphophorus helleri*. — Fig. 27. M I of *Mollienisia sphaenops*. — Fig. 28. M II of *Mollienisia sphaenops*. — Fig. 29. M I of *Mollienisia sphaenops* var. *melanistica*. — Fig. 30. M I of *Mollienisia velifera*. — Fig. 31. M I of *Mollienisia sphaenops* × *M. velifera*. — Fig. 32. M I of *Phalloceros caudimaculatus* var. *reticulatus*. — Fig. 33. M II of *Phalloceros caudimaculatus* var. *reticulatus*. — Fig. 34. M II of *Heterandria formosa*. — Fig. 35. M I of *Rivulus santensis*. — Fig. 36. M I of *Mollienisia sphaenops* var. *melanistica* × *M. sphaenops*. 4 univalents lying nearer the pole than the 19 bivalents are marked by arrows.

able to detect any secondary constrictions. This may possibly be due to the extremely small size of the chromosomes, or to the nucleolus organizers of these species being terminal.

As regards the duration of the different stages of the mitosis I

have naturally been unable to make any direct measure of the time for these, but I have estimated their relative duration by counting the number of cells in different stages. The counting was made on smear preparations of 6 *Lebistes* embryos in a stage of about 12 somites.

	Prophase	Metaphase	Anaphase	Telophase	Total
Number of cells	112	91	3	103	309
%	36,4	29,3	1,0	33,3	100,0

Particularly striking is the extremely short duration of the anaphase. Any more intimate somatic pairing is very likely unable to develop during the anaphase, contrary to what is the case in Salmonidae (SVÄRDSON, 1941). Its maximum may instead lie in telophase.

In addition it is to be noted how the degree of contraction in the chromosomes increases through the spermatogenesis and by and by becomes as high as at meiosis.

In one case I found a tetraploid cell at prophase. The species was *Mollienisia sphaenops* var. *melanistica*. Concerning the origin of this cell I consider syndiploidy to be most probable. Similar cases have been discovered in Salmonidae by SVÄRDSON (1941) in somatic tissue and by the present writer in many species of Amphibia (unpubl.).

3. MEIOSIS.

The meiosis also shows great conformity with the normal scheme. During zygotene a very strong polarization of the ends of the chromosomes is taking place, while the centre parts hang out in the nucleus as long loops. Especially in CARNOY-fixed material this appears with unprecedented clarity, but this fixative seems to evoke the polarization of both leptotene and pachytene. Therefore I consider this strong polarization as an artefact caused by unsuitable fixation.

Among previous investigators, WINGE (1922) and DILDINE (1936) deny the existence of this stage in *Lebistes*, while VAUPEL finds it in the same way as I described it. RALSTON describes it in Xiphophorini.

Pachytene is a very distinct stage and is of very long duration. A weak polarization distinguishes it.

There is no growth stage between the second spermatogonia and the spermatocytes in Cyprinodontidae as in Dipnoi and Amphibia. Therefore the spermatocytes are extremely small as in all Teleosts. The same thing is pointed out in Salmonidae by JONES (1939).

The nuclei become diffuse and granular at diplotene but soon clear up, whereupon the diakinesis becomes very evident. How WINGE (1922) could overlook the stage is inexplicable to me. In *Lebistes* it is certainly very short but still sufficiently usual to be found in every testis I have examined. The chromosomes lie in typical diakinetic figures with mainly terminalized chiasmata.

The chromosomes go synchronously into the metaphase plate except in the cases where univalents occur. In no species have I found any chromosome pair that constantly lags behind the others in the formation of the plate. My results coincide therefore with FRIEDMAN and GORDON's (1934) and with WINGE's and IRIKI's but are in opposition to RALSTON's and VAUPEL's. This problem will be dealt with further on in the chapter on the sex chromosomes.

As at mitosis the chromosome numbers at meiosis of the species examined by me correspond with those previous writers have found, as far as the species have been examined before. The single exception is *Mollienisia sphaenops* and also to some degree *Platypoecilus variatus*.

Concerning the last-mentioned species I must unfortunately admit that I have not succeeded with absolute certainty in establishing its chromosome number. No fixative used has given satisfactory results, although I have also used BENDA's and CHAMPY's fluids. The chromosomes of the metaphases of meiosis always clump together and become diffuse and impossible to examine. So much I can, however, with all certainty state, that no univalents with an appearance and behaviour characteristic of them are found in my material. All chromosomes always lie in the plate and no lagging, etc. occur either at the formation of the metaphase plate or at anaphase. FRIEDMAN and GORDON (1934) found in their material that one chromosome at the metaphase of the first division is smaller than the others and »evidently univalent». The number should therefore be $2 \times 24 + 1$. If this should be the actual condition it is extremely remarkable that this univalent invariably lies in the plate. It further involves certain cytogenetical peculiarities. If the univalent appears in both sexes and always as a univalent, a selective mortality must take place. If, however, the univalent is present only in the male, it must be either an X-chromosome or an accessory Y-chromosome. The first assumption, implying that the animal is of the XO-type, must be erroneous, inasmuch as KOSSWIG confirms by genetical means that the male of *Pl. variatus* has XY. The other might be accepted in so far as the conditions in the cross with *Pl. maculatus* might indicate that a fragmentation has taken place. One of the

Pl. variatus chromosomes + the univalent is homologous with one of the chromosomes of *maculatus*.

As is seen, these possibilities are affected by the fault that they require certain additional assumptions which, although not impossible in themselves, are yet rather incredible and in any case unsubstantiated. None of them can either explain the peculiar fact that »the univalent» always lies in the plate and passes into it synchronously with the bivalents. Although I am unable to determine the chromosome number with absolute certainty, I am inclined to doubt the existence of the univalent. Whereas no reason exists to doubt the number of units FRIEDMAN and GORDON gave, it seems likely to me that the small chromosome which these authors interpret as a univalent is in reality a small bivalent. The chromosome number of *Platypoecilus variatus* should therefore be n = 25.

The conditions of *Mollienisia sphaenops* have already been discussed in the chapter »Mitosis».

After the first telophase a short resting stage follows. The interphase is often (*Limia*) so short that both divisions can be found in the same lobe, in other cases (*Lebistes*) it is somewhat longer. In conformity with VAUPEL but in contrast to WINGE I have observed a cell wall being formed between the first and the second divisions.

The second division takes place fully normally. No lagging, etc. has been observed. Where the numbers could be established they always corresponded with those in the first division. Exceptions are naturally the undermentioned abnormal species. Where univalents are found they always divide at the second division.

After the second division the transformation takes place of the spermids to spermatozoa, which, however, is outside the scope of this examination.

4. ABNORMITIES.

A. POLYPLOIDY.

In one case (in *Xiphophorus helleri*) I have observed two tetraploid cells at diakinesis. The cells in question are considerably larger than the diploids lying around (Fig. 26). In one of them I could count 46 units, in the other I did not succeed with any degree of certainty in arriving at an estimate of the number. Whether in the first one the number was 48 bivalents, two of which being concealed by others and so eluding me in the counting, or whether multivalents were possibly formed, I was unable to ascertain. The origin of these two cells can

naturally not be stated with absolute certainty, but I hold syndiploidy to be the most feasible explanation. It is interesting to note that very likely none, and in any case not more than two multivalents, have been developed in this cell. This may be an indication that the chiasma frequency in this species is extremely small. This agrees very well with the observations of the diakinesis in all examined species.

B. DEGENERATION.

Another striking abnormity is the large number of dead and degenerated cells in the testes of these animals. The degeneration seems

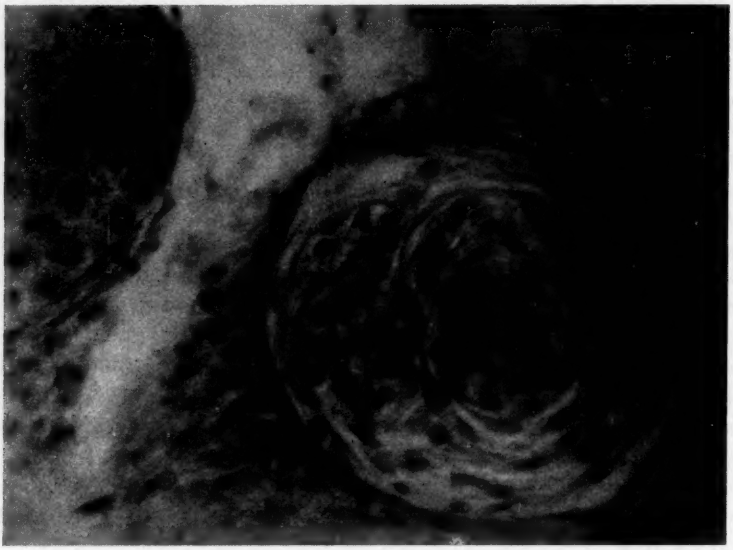

Fig. 37. Residual body in the testis of *Glaridichtys falcatus*. — 600 ×.

most often to affect one of the middle spermatogonial generations, and entire lobes may then show characteristic degeneration symptoms. The chromatin is clustered together in intensely stainable structureless clumps, which lie accumulated in the periphery of the testis. Where the first division is abnormal and contains univalents, the death rate among spermids is very high.

C. SEX REVERSAL.

Special degeneration phenomena are found in animals which go through or have gone through sex reversal. Sex reversal is known in

most Cyprinodonts (REGNIER, 1938) and always seems to proceed from the feminine to the masculine stage. It may take place in all stages of development, but naturally leaves more definite traces when an already fully developed female reverses to a male. The ovarium then degenerates completely, the eggs are capsuled and affected by phagocyting cells. In certain cases they might even spread to, and also be capsuled and phagocyted in, other organs such as the liver, intestines, heart, etc. After a time so-called residual bodies are developed. I have found residual bodies in *Xiphophorus helleri*, *Glaridichtys falcatus* (Fig. 37) and the hybrid *X. helleri* × *Pl. maculatus*. Sex reversal in *Glaridichtys* has so far as I know not been previously detected.

Considerably less are the changes which follow the reversal from females to males in early embryonic stages, which is very common and may be normal in many species. This so-called juvenile hermaphroditism has been described before in several species (BREIDER, 1935; DILDINE, 1936; KOSSWIG, 1935 a; REGNIER, 1938).

D. MOLLIENISIA SPHAENOPS VAR. MELANISTICA WICKBOM.

This form, which according to INNES (1935) and ARNOLD and AHL (1936) is an entirely black melanistic form of *Mollienisia sphaenops* developed through lengthy selection and systematic race-crossings, has on examination according to REGAN's key also shown itself to belong to that species. On account of its deviating chromosome number I think, however, that it should be considered as a variety, differing by its black colour, which even appears in the pigmented parts of the eye, and also by its chromosome number, which deviates from the main species. I propose for it the name var. *melanistica*.

It crosses easily with the common *Mollienisia sphaenops*, and the hybrids are black and grey spotted.

The variety *melanistica*, usually called »Black Molly», should be carefully distinguished from similar black forms of *M. latipinna* and *M. velifera*. Cytologically it deviates pronouncedly from the main species. The mitotic metaphase plates contain 38 chromosomes of usual Cyprinodont appearance and in typical somatic pairing (Fig. 9). Meiosis shows several peculiarities. Metaphase I most often ($^2/_3$ of all cases) shows 19 bivalents (Fig. 29). In such cells the division proceeds fully normally, the second division also, and the result is 4 spermids with n = 19. On the other hand, in the cases ($^1/_3$ of all cases) where the pairing at metaphase is not complete but univalents are developed, the division takes place abnormally. The univalents,

to a number of between 2 and 10, disperse freely in the nucleus but may at late metaphase arrange themselves into the plate. The plate will then consist of 18 bivalents and 2 univalents, 17 bivalents and 4 univalents, etc. At anaphase there exists no centromere repulsion driving the univalents to the poles. Instead they remain in the plate till the stretching of the spindle takes place. Then they follow passively along and are distributed at random to the poles. In the cases where this distribution of lagging univalents could be observed in the microscope, I have found the following numbers:

To one pole	To the other pole	% of observed anaphases	Number of normal anaphases
1	0	13,5	
1	1	3,5	
2	0	7,0	
2	1	5,5	
2	2	0,7	
3	0	1,4	
3	1	0,7	
3	2		
3	3	1,0	
5	1		
		33,3 %	222 = 66,6 %

The table takes into consideration only the univalents which at the respective anaphases could be clearly distinguished as such. Furthermore, naturally, an unknown number might be present hidden in the multitude of bivalents moving in the direction of either pole. This of course is the case in all the cells which in the table appear to have an unequal number of univalents (the categories »1—0», »2—1», »3—0», etc.).

The high death rate among the cells at interphase of this animal may probably be attributed to this distribution of univalents and may have its explanation in the fact that cells with chromosome numbers differing too much from the normal are not viable.

The second division appears to take place normally, but during earlier spermid stages a number of cells degenerate. Probably these are, among others, the descendants of cells which at the first division got combinations of chromosomes which to some, though not so high, degree differ from the normal one. All spermatozoa must have the chromosome number 19.

Concerning the reasons for all these anomalies, which are plainly all connected with deficient pairing at diplotene, diakinesis and metaphase, it is naturally difficult to give any opinion. When the cells are so small and the chromosomes so many as in Cyprinodontidae, an analysis of the pachytene figures is almost impossible, but I believe I am able to state that at pachytene all the chromosomes show complete pairing. Probably the chiasma frequency in this form is so low that there is nothing to hold the chromosomes together at diplotene, diakinesis and metaphase, and therefore they fall apart and form univalents. The hybrid *Mollienisia sphaenops* var. *melanistica* × *M. sphaenops* gives a further proof of the probability of this theory. The hybrids are of both sexes, but the females are in the majority. (In one litter observed by me 19 : 1). At the spermatogonial metaphases there are in the hybrid 42 chromosomes at least partly in somatic pairing (Fig. 10). The members of one pair, on the figure marked with arrows, are not morphologically alike. Neither do they show the same degree of somatic pairing as the others. At the metaphase of meiosis 19 bivalents always lie in the plate (Fig. 36). There are a number of univalents as well, 1—4. These lie sometimes in the plate, sometimes outside it, and they show the same behaviour as I have reported in *M. sph.* var. *melanistica*. The majority of the spermids therefore have 19 chromosomes; those having one or more in addition will probably degenerate. This assumption is confirmed by the fact that so many secondary spermatocytes and spermids revert to degeneration.

The hybrid has plainly received 19 chromosomes from its mother and 23 from its father. Of these, evidently 18 from each of the parents are completely homologous. One chromosome from the father and one from the mother are, moreover, probably so homologous that they show full pairing at meiosis, though the mitotic pictures show that they are morphologically different. The four remaining *sphaenops* chromosomes are included in the meiosis as univalents and distributed haphazardly on the second spermatocytes.

What possibilities do these cytological observations disclose for the explanation of the origin of *M. sphaenops* var. *melanistica*? Plainly a certain elimination of chromosomes has taken place which has affected four of the chromosomes of the main species. Moreover, another of these has had its morphology changed through translocation or other structural change. Whether all these alterations have taken place simultaneously or gradually is now impossible to determine, as no data whatever can be obtained about the origin of the species

beyond the fact that it was produced in America about 1935 (INNES, 1935).

If, according to MEYER (1938), the American aquarium variety of *M. sphaenops* should have the chromosome number n = 18, 2n = 36, one would have to assume that var. *melanistica*, if it has the previously mentioned American form as origin, has arisen through a double fragmentation, which should explain the great number of cells with complete pairing at meiosis. On the other hand, it is difficult to imagine how this fragmentation could have taken place in a set of only rod-shaped chromosomes so that all four fragments developed from one chromosome pair should get centromeres. Another unaccountable feature is the pairing between the 19 chromosomes of the *melanistica* and 19 of my *sphaenops* variety's 23. One can of course strain an explanation whereby my form would have still more fragmented chromosomes (still all with centromeres!). It seems a strange decree of fate that this fragmentation should be the cause of exactly that chromosome number which distinguishes most Cyprinodonts, except Xiphophorini, and especially *Mollienisia velifera* and the genus *Limia*, which is closely related to *Mollienisia*.

As was mentioned before, *Mollienisia velifera* also has a black variety. I have not succeeded in obtaining it in a pure form, but I have examined an F_1-male of the crossing between a black female and a normal *velifera* male. The hybrid was black-speckled, in the same manner as corresponding hybrids of *M. sphaenops*, and in appearance very much the same as a normal *velifera* male, though smaller, but not so small as the black form. The chromosome number at metaphase I of this hybrid was 23, and therefore in all probability the black variety of *M. velifera* has the same number as the main species. On account of lack of material I have not been able to examine the mitosis, but nothing indicates that deviations from the main species should be expected. The meiosis is completely normal.

E. LIMIA CAUDOFASCIATA VAR. TRICOLOR.

Also *Limia caudofasciata* var. *tricolor* STOYE (*L. dominicensis* REGAN, *L. melanogaster* GUENTHER) sometimes has some univalents at the first spermatocyte division. More than two I never observed. They are seen in about 6 % of all cells, but I do not think that those daughter cells and spermids which for these reasons get deviating chromosome numbers will be viable. I have not found any deviating chromosome number in any individual of this species. In this case,

too, the explanation that this is due to low chiasma frequency seems to be the most probable.

F. HYBRIDS.

I find it highly remarkable that the hybrids between species of Cyprinodontidae with the same chromosome number hitherto investigated all have complete pairing at meiosis. FRIEDMAN and GORDON (1934) found normal meiosis without univalents in all Xiphophorini hybrids they examined; RALSTON (1934) made the same observations in his *Xiphophorus-Platypoecilus* hybrids, and I have found regular meiosis in the hybrids *Xiphophorus* × *Platypoecilus* and *Mollienisia sphaenops* × *M. velifera*. This implies that every chromosome in these species must have homologous parts with those in the other species, parts which must be sufficiently large to allow the chromosomes to form chiasmata. This fact has some consequences for the relationships between those species which must be separated mainly by small genetical divergences. This will be the object of further investigations.

5. THE SEX CHROMOSOMES OF CYPRINODONTIDAE AND OF PISCES IN GENERAL.

A short previous report on the contents of this chapter has already been published (WICKBOM, 1941).

The problem of sex chromosomes of Cyprinodontidae has engaged the attention of several investigators. The few cytological results hitherto obtained give very meagre information about the sex chromosomes of this group as of Teleosts in general. Certain authors (GEISER, 1924; FOLEY, 1926; VAUPEL, 1929; RALSTON, 1934; BENNINGTON, 1936; BARIGOZZI, 1937) find conditions at meiosis which, according to them, would indicate the presence of heterochromosomes in Teleosts. RALSTON's opinion is the most advanced in that he claims to have found sex chromosomes with characteristic behaviour at the meiosis of the whole group Xiphophorini. The other authors have only sometimes found »lagging», etc. The same peculiarities are found by BENNINGTON in *Betta splendens* and by BARIGOZZI in *Cyprinus*. I cannot endorse the opinions of RALSTON, BENNINGTON and BARIGOZZI, but concerning Xiphophorini I agree with FRIEDMAN and GORDON (1934) that lagging, etc. sometimes occurs but not as a rule.

As regards *Betta*, SVÄRDSON and the present writer have not been able to verify BENNINGTON's assertions (SVÄRDSON and WICKBOM, 1942).

There appears to me to be a more credible explanation of the »irregularities» sometimes found at early anaphases than those given by RALSTON, BENNINGTON and others. These »irregularities» must depend upon the varying lengths and chiasma numbers of the chromosomes. In reality anaphase stages are found where all small chromosomes have reached the poles, while the larger ones almost touch each other with their ends. The distance between the centrosome and the centromeres of the chromosomes passing to the same pole is, however, almost equal.

RALSTON's opinion that one pair of large chromosomes which at the meiotic metaphase lie apart from the others, because of this position, should represent the sex chromosomes, need not be correct in any way. Rather does it lie in the nature of things, besides according with the theories of DARLINGTON (1937) and others respecting the forces affecting the chromosomes during division, that the repulsion which in a metaphase plate rules between the bivalents must be proportional to the size of the surfaces of the chromosomes. This, then, is the reason why the small chromosomes lie in the centre of a metaphase plate, while the larger ones are arranged at the periphery. In most figures published by FRIEDMAN and GORDON as well as in most of mine it is evident that one or two large chromosome pairs are lying some distance away from the others. This does not signify that the chromosomes in question are the sex chromosomes, but, as shown, is explained as a function of their size. Thus, according to my opinion, the two phenomena which certain previous investigators have interpreted as an indicium of cytologically discernible sex chromosomes of Teleosts, depend instead upon the simple fact that one pair of chromosomes is larger than the others. All these investigators state, too, that the largest pair represents the sex chromosomes. Of course there is nothing to prevent them from being sex chromosomes as well as any other pair, but »their characteristic behaviour during division» is not a function of their possible quality but probably of their size only. The peculiarities which distinguish the sex chromosomes especially of Mammalia and Insecta are of course not *sensu stricto* caused by these chromosomes functioning as sex determinators either, but only by the changes in structure which they have undergone partly in consequence of non-homologies between the sex-determinating genes.

As was shown, the same peculiarities often distinguish other chromosomes which for some reason consist largely of heterochromatin, as certain fragments, etc. (DARLINGTON, 1937), or which for some other reason have a lower chiasma frequency than others in the same set,

or which differ from the others in respect of the relation between the number of terminal and interstitial chiasmata. When, as in the case of Cyprinodontidae and probably the whole order Teleostei, the homology between X- and Y-, W- and Z-chromosomes is so great that at the mitoses they cannot be distinguished from each other, and when the staining capacity of different chromosomes gives no support to the opinion that any of the chromosomes should be heterochromatic, there is no reason to expect special behaviour of the sex chromosomes. It is a violation against nature to evoke such behaviour by interpreting every irregularity of a division as produced by a pair of heterochromosomes.

Hitherto there is no cytological evidence for the presence of heterochromosomes in Teleosts. Results obtained by genetical work, however, show that these animals have a sex-linked inheritance with chromosome mechanisms of *Drosophila* and *Abraxas* type. Moreover, some of them have a phenotypic sex determination. All these facts support KOSSWIG's (1935 a and b) and WINGE's theories of the heterochromosomes of this group, based upon genetical work. The homology between the sex chromosomes is in reality so great that pairing and crossing over occur between them (WINGE, 1923; BELLAMY, 1928). The homology between the sex chromosomes of different species of Xiphophorini is evidently very extensive (KOSSWIG, 1936). The sex chromosomes of the egg-laying Cyprinodontidae are of the same kind as those of the viviparous forms. Genetical crossing over is described by AIDA (1936 *et al.*), and I was unable to find cytological evidence of sex chromosomes in *Rivulus santensis*, as was also IRIKI (1932) in *Aplocheilus*. A continued investigation of the sex chromosomes in different groups of Teleosts would doubtless give some valuable points of view on the problem of the evolution of the heterochromosomes of Vertebrata, which may in turn throw fresh light upon the more advanced and complicated conditions met with among Reptilia, Aves and Mammalia.

The opinion that the heterochromosomes in Pisces and even in Amphibia are in the beginning of the evolution that those of Amniota have passed through has been presented by MAKINO (1933 and 1939) and GALGANO (1941 *et al.*). No explanation of the lagging and precociousness in certain species claimed by several authors can, however, be given by MAKINO, except that they might be caused by unsatisfactory fixation, which of course may often be the case. The theory propounded here explains these in a more satisfactory way, at the same time as it takes into account the genetic results gained within different genera.

MAKINO's previously mentioned explanation would be valid to a high degree in regard to the cytological observations which BARIGOZZI (1937) has made on *Cyprinus carpio* var. *specularis* (2n = 19 ♂). Different varieties of *Cyprinus carpio*, among them also *specularis*, have curiously enough also been examined by MAKINO (1939) (2n = 104 ♂), though this investigator does not seem to have seen BARIGOZZI's work, and hence he does not discuss the difference between his own and BARIGOZZI's results.

Acknowledgements. — The author takes this opportunity to express his sincere thanks to his teacher, Professor GERT BONNIER, for valuable advice and criticism during the work; to the Director of the Department of Developmental Physiology and Genetics, Wenner-Gren's Institution, Professor SVEN HÖRSTADIUS, for placing at his disposal laboratories, material and help in many respects; to the technical assistant of the Department, Miss KERSTIN BACHMAN, for preparations and microphotography; and especially to his friend and collaborator Mr. GUNNAR SVÄRDSON for help and criticism during the investigation.

SUMMARY.

1. The regular mitosis and meiosis of Cyprinodontidae are described. Somatic pairing is always found at mitosis.

2. The chromosome numbers of 16 species, subspecies and hybrids are given.

3. Some abnormities (polyploidy, sex reversal, degeneration) are described.

4. The sex chromosomes of Cyprinodontidae, where they exist, probably like those of all Teleosts hitherto investigated, are not distinguished by particular behaviour at meiosis owing to their being sex chromosomes, but diverge in no respect cytologically from the autosomes. This is probably due to their slight differentiation, which may be traced back to the low systematic position of the Cladus in the Vertebrate system. The statements of previous investigators in this respect are explained in another way on the basis of other cytological facts.

LITERATURE CITED.

1. AIDA, T. 1936. Sex reversal in *Aplocheilus latipes* and a new explanation of sex differentiation. — Genetics 21: 36—153.

2. Arnold, J. P. and Ahl, E. 1936. Fremländische Süsswasserfische. — Braunschweig.
3. Barigozzi, C. 1937. La gametogenesi e la sessualitá di *Cyprinus carpio* var *specularis*. — Soc. It. Sci. Nat. Mus. Milano Atti 76: 88—104.
4. Bellamy, A. W. 1928. Color pattern inheritance and sex in *Platypoecilus maculatus*. — Genetics 13: 226—232.
5. Bennington, N. L. 1936. Germ cell origin and spermatogenesis in the Siamese fighting fish, *Betta splendens*. — Journ. of Morph. 60: 103—125.
6. Breider, H. 1935. Geschlechtsbestimmung und -Differenzierung bei *Limia nigrifasciata, caudofasciata, vittata* und deren Artbastarden. — Zeitschr. f. ind. Abst.- u. Vererbungsl. 68: 265—299.
7. — 1939. Untersuchung zur Rassen-, Art- und Gattungsdifferenzierung lebendgebärender Zahnkarpfen. — Verh. Dtsch. Zool. Ges. E. V. (Zool. Anz. Suppl. 11—12), 40—41: 221—237.
8. Darlington, C. D. 1937. Recent advances in cytology. 2nd edition. — London.
9. Dildine, G. C. 1936. Germ cell origin and gonad differentiation in the viviparous top-minnow *Lebistes reticulatus*. — Journ. of Morph. 60: 261—277.
10. Essenberg, J. M. 1923. Sex differentiation in the viviparous teleost *Xiphophorus helleri*. — Biol. Bull. 45: 46—97.
11. Foley, J. O. 1926. The spermatogenesis of *Umbra limi*, with special reference to the behaviour of the spermatogonial chromosomes and the first maturation division. — Biol. Bull. 50: 117—147.
12. Fraser, A. C. and Gordon, M. 1928. Crossing over between the W- and Z-chromosomes of the killifish *Platypoecilus*. — Science 57: 470.
13. Friedman, B. and Gordon, M. 1934. Chromosome numbers in Xiphophorin fishes. — Amer. Nat. 68: 446—455.
14. Galgano, M. 1941. La variazione dei chiasmi nei maschi e negli intersessuati di »*Rana esculenta*» L. — Arch. Ital. Anat. e Embr. 66: 127—164.
15. Geiser, S. W. 1924. Sex ratios and spermatogenesis in the top-minnow *Gambusia holbrooki*. — Biol. Bull. 47: 175—212.
16. Gordon, M. 1937. Inheritance of sex and crossing over in the sex chromosomes in the Platyfish. — Genetics 22: 376—392.
17. Innes, W. T. 1935. Exotic aquarium fishes. — Philadelphia.
18. Iriki, S. 1932. Preliminary notes on the chromosomes of fishes. — Proc. Imp. Acad. Tokyo. 8: 262—263.
19. Jones, J. W. 1939. Histological changes in the testis in the sexual cycle of male salmon parr (*Salmo salar* L. juv.). — Proc. Roy. Soc. London, Ser. B. 128: 499—509.
20. Kosswig, C. 1931. Die geschlechtliche Differenzierung bei den Bastarden von *Xiphophorus helleri* und *Platypoecilus maculatus* und deren Nachkommen. — Zeitschr. f. ind. Abst.- u. Vererbungsl. 57: 227—305.
21. — 1935 a. Die Kreuzungen zweier XX- bzw. XY-Geschlechter mit einander. — Der Züchter 7: 40—48.
22. — 1935 b. Genotypische und phänotypische Geschlechtsbestimmung bei Zahnkarpfen. V. — Roux' Arch. 133: 118—140.
23. — 1936. Nicht homologe Heterochromosomen bei nächstverwandten Arten. — Biol. Zbl. 56: 199—207.

24. KOSSWIG, C. 1937. Weiteres über die Homologieverhältnisse der Heterochromosomen bei Zahnkarpfenarten, die mit einander kreuzbar sind. — Biol. Zbl. 57: 38—47.
25. MAKINO, S. 1933. The chromosomes of the sticklebacks *Pungitius tymensis* and *P. pungitius*. — Cytologia 5: 155—168.
26. — 1939. The chromosomes of the carp, *Cyprinus carpio*, including those of some related species of Cyprinidae for comparison. — Cytologia 9: 430—440.
27. MEYER, H. 1938. Investigations concerning the reproductive behaviour of *Mollienisia »formosa»*. — Journ. of Gen. 36: 329—367.
28. RALSTON, E. M. 1934. A study of the chromosomes of *Xiphophorus*, *Platypoecilus* and *Xiphophorus* × *Platypoecilus*-hybrids during spermatogenesis. — Journ. of Morph. 56: 423—433.
29. REGAN, C. T. 1913. A revision of the Cyprinodont fishes of the subfamily Poeciliinae. — Proc. Zool. Soc. London, 26: 977—1018.
30. REGNIER, M.-TH. 1938. Contribution à l'étude de la sexualité des Cyprinodontes vivipares. — Bull. Biol. France Belg. Tome 72, Fasc. 4: 385—493.
31. SVÄRDSON, G. 1941. Somatic pairing in *Salmo* and *Coregonus*. — Ark. f. Zoologi. Bd 33 B, N:r 9: 1—6.
32. SVÄRDSON, G. and WICKBOM, T. 1939. Notes on the chromosomes of some Teleosts. — Hereditas XXV: 472—476.
33. — and — 1942. The chromosomes of two species of Anabantidae (Teleostei), with a new case of sex reversal. — Hereditas XXVIII: 212—216.
34. VAUPEL, J. 1929. The spermatogenesis of *Lebistes reticulatus*. — Journ. of Morph. 47: 555—587.
35. WICKBOM, T. 1941. The sex chromosomes of Cyprinodontidae and of Teleosts in general, with a list of new chromosome numbers in Cyprinodontidae. — Ark. f. Zoologi. Bd 33 B, N:r 10: 1—6.
36. WINGE, Ö. 1922 a. A peculiar mode of inheritance and its cytological explanation. — Journ. of Gen. 12: 137—144.
37. — 1922 b. One-sided masculine and sex linked inheritance in *Lebistes reticulatus*. — Journ. of Gen. 12: 145—162.
38. — 1923. Crossing-over between X- and Y-chromosomes in *Lebistes*. — Journ. of Gen. 13: 201—217.
39. — 1927. The localisation of eighteen genes in *Lebistes reticulatus*. — Journ. of Gen. 18: 1—43.
40. — 1930. On the occurrence of XX-males in *Lebistes*, with some remarks on AIDA's so-called »non-disjunctional» males in *Aplocheilus*. — Journ. of Gen. 23: 69—76.
41. WITSCHI, E. 1934. In ALLEN's »Sex and internal secretions» (pp. 198—207). — Baltimore.

DIE ENTWICKLUNG DES EMBRYOSACKS UND DIE BEFRUCHTUNG BEI POA ALPINA

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(With a Summary in English)

EINLEITUNG.

VON MÜNTZING wurde festgestellt, dass *Poa alpina*-Pflanzen aus verschiedenen Teilen von Schweden apomiktische Samenbildung haben, während gewisse Pflanzen aus der Schweiz sexuell waren (MÜNTZING, 1933, 1940). Alle Typen haben gute Pollenkörner, und Bastarde zwischen Sexuellen und Apomikten konnten hergestellt werden; sie waren immer sexuell. Bei einer Apomikte aus Pajala wurde die EMZ [1] nicht geteilt, sie bildete direkt den ES nach dem *Antennaria*-Schema. ÅKERBERG (1942) fand weiter eine mitotische Kernteilung in der EMZ einer Apomikte aus Bayern. In *Poa alpina* kommt also Diplosporie vor, nähere Untersuchungen sind aber nicht gemacht worden.

Apomixis kommt aber in anderen *Poa*-Arten vor. KIELLANDER (1935, 1937) hat früher Diplosporie bei *P. nemoralis*, *palustris* und *glauca* festgestellt. Es teilte sich aber bisweilen der Kern der EMZ durch Meiosis, und Makrosporen wurden gebildet. Sehr eingehend ist die einer anderen Sektion der Gattung zugehörige *P. pratensis* untersucht worden, in der MÜNTZING Apomixis entdeckt hat (1933). Hier kommt aber Aposporie vor (TINNEY, 1940; ÅKERBERG, 1942 und frühere Abhandlungen; KIELLANDER, 1941, 1942). Die sexuellen Typen von *P. pratensis* bilden den ES aus einer haploiden Makrospore, bei den apomiktischen Typen degeneriert die EMZ oder die Makrosporen, und der ES (oder die ES-e) wird (oder werden) von Nuzelluszellen gebildet. Die apomiktischen Typen von *P. pratensis* sind pseudogam, ohne Pollination werden keine reifen Samen gebildet.

Die Erscheinung der Pseudogamie (= durch Pollination induzierte Agamospermie) bei den Blütenpflanzen ist nach den Untersuchungen von NOACK über *Hypericum perforatum* in eine ganz neue Beleuchtung gelangt (1939). Der haploide ES degeneriert bei *H. perforatum* in der Regel, ein aposporischer ES wird entwickelt, die Eizelle dieses ES bildet

[1] EMZ = Embryosackmutterzelle, ES = Embryosack.

ohne Befruchtung Embryo, der Zentralkern muss aber befruchtet werden, um Endosperm bilden zu können. Die Spermakerne wurden nicht beobachtet, Chromosomenzählungen im Embryo und Endosperm nach Selbstung und nach Kreuzung mit *H. quadrangulum* haben aber diese Entwicklung sichergestellt. Das Wesen der Pseudogamie bei *H. perforatum* ist also, dass die ohne Befruchtung gebildeten Embryonen sich nicht ohne Endosperm vollständig entwickeln können, und dass das Endosperm erst nach der Befruchtung des Zentralkerns entsteht. In *H. perforatum* ist die Grenze zwischen Sexualität und Apomixis auch in der Hinsicht unscharf, dass die Eizelle des aposporischen ES befruchtet werden kann.

Eine Übersicht von Parthenogenesis und Pseudogamie im Lichte alter und neuer Untersuchungen haben GENTCHEFF und GUSTAFSSON (1940 b) gegeben. Sie machen es klar, dass die Pseudogamie in *Potentilla* eine Befruchtung des Zentralkerns des aposporischen ES ist, ohne Endosperm wird die Embryobildung begonnen, aber nicht abgeschlossen. In *Rubus* kommt wahrscheinlich diese Fortpflanzung auch vor (GUSTAFSSON, 1942). In *Poa pratensis* fand ÅKERBERG (1942), dass die Embryobildung früh beginnt, der Zentralkern teilt sich viel später, sehr wahrscheinlich erst nachdem er befruchtet worden ist. Diese Pseudogamie scheint also gewöhnlich zu sein. Dass in *P. pratensis* wie in *Hypericum perforatum* die Eizelle des aposporischen ES befruchtet werden kann, zeigt das schon früher von ÅKERBERG und MÜNTZING festgestellte Hervorgehen triploider Varianten aus apomiktischen Formen. Selten kommen auch haploide Varianten vor, dies muss darauf beruhen, dass eine Makrospore sich bisweilen zum fertigen ES entwickelt und parthenogenetisch einen Embryo entwickelt (ÅKERBERG, l. c.). KIELLANDER (1942) fand gar eine zweimalige Halbierung der Chromosomenzahl. Eine Pflanze mit 72 Chromosomen gab einen Samen mit Drillingen, von diesen hatten zwei 72 Chromosomen, der dritte war subhaploid und hatte nur 18. Einen haploiden Embryo hat er in *P. palustris* beobachtet (1935).

Professor ARNE MÜNTZING bot mir an, seine *Poa alpina* embryologisch zu untersuchen. Ich wollte auch gern versuchen, Präparate über das Vorkommen und Verhalten der Spermakerne im ES der apomiktischen Typen zu bekommen. Die Entwicklung einiger sexuellen Typen wurde auch untersucht, wie auch einige Bastardpflanzen aus der Kreuzung Sexuell × Apomikt. Professor MÜNTZING hat in freundlicher Weise meine Arbeit unterstützt.

Es wurden Fixierungen von 12 Pflanzen von 11 verschiedenen

Klonen gemacht. Im Jahre 1940 wurden die Feldpflanzen in Svalöf fixiert, 1941 wurden dieselben Pflanzen eingetopft und standen im Fenster meines Speisezimmers. Die Anthese war 1941 oft nicht so gut, besonders selten öffneten sich die Antheren bei der Gotland-Apomikte. Die oft schwache Pollination erwies sich aber in gewisser Hinsicht als vorteilhaft, die meisten Beobachtungen sind an diesem Material gemacht. Die erste Entwicklung der Samenanlagen konnte nicht mehr eingehend studiert werden, weil die Fixierungen zu spät gemacht wurden; bei den Sexuellen geschieht die Teilung der EMZ sehr früh.

Wie bei anderen *Poa*-Arten sind die Samenanlagen bitegmisch, das innere Integument ist am besten entwickelt und bildet den kurzen Mikropylenkanal, beide Integumente sind zweischichtig. Bisweilen war die Entwicklung etwas gehemmt, so dass auch das innere Integument nicht über den Nuzellusscheitel emporwuchs, und infolgedessen kein Mikropylenkanal gebildet wurde. Die Pflanze 91 zeigte stärkere Störungen in der Integumentbildung. Einmal war z. B. das äussere Integument sehr kräftig entwickelt: auf der einen Seite der noch jungen Samenanlage war es hoch über den ungewöhnlich breiten Nuzellusscheitel aufgewachsen, auf der anderen Seite quer über ihn gewachsen. Eine zweite abnorme Samenanlage zeigte die Anlegung eines dritten Integuments. Der Nuzellus ist gross, hat immer nur eine EMZ, die nicht durch Deckzellen von der Epidermis getrennt ist, der Nuzellus ist also syndermal.

DIE SEXUELLEN SIPPEN.

Die sexuellen Pflanzen aus der Schweiz hatten verschiedene Chromosomenzahlen. Es wurden fünf Sippen untersucht, die fixierten Pflanzen, und die von MÜNTZING festgestellten Chromosomenzahlen sind:

45	$2n = 25$	90	$2n = 23$	111	$2n = 31$
57	$2n = 22$	91	$2n = 24$		

Von diesen ist 91 als Mutterpflanze zu der Kreuzung Sexuell × Apomikt verwendet.

Die erste Entwicklung geht sehr rasch, die Teilung der EMZ ist viel früher als die der PMZ in den Antheren. Die in Fig. 1 abgebildete junge Makrosporentetrade ist von einer Blüte, deren PMZ frühe Pachytäne zeigten. Die EMZ wurde nur einige Male beobachtet, sie ist recht gross, ihre Form scheint zu wechseln. In 91 wurde sowohl eine niedrige aber sehr breite, wie eine längere EMZ gesehen. In allen Pflanzen

wurden Makrosporen gebildet, die Teilung der EMZ wurde doch nicht beobachtet (vergl. aber S. 45). Nach ÅKERBERG und KIELLANDER sind die Makrosporen in *P. pratensis* und *palustris* linear angeordnet, und die Teilung der mikropylaren Dyadenzelle ist verspätet und unterbleibt sehr oft, so dass nur drei Makrosporen gebildet werden. Lineare Tetraden kommen auch hier vor (vergl. Fig. 32). Die mikropylaren Makrosporen können rasch degenerieren und vom sterilen Nuzellusgewebe verdrängt werden, oder richtiger, der von ihnen eingenommene Raum wird nach ihrer Degeneration durch das Zusammenkommen von sterilem Gewebe ausgefüllt (Fig. 6). Dies ist die Ursache, dass man oft zwei tief begrabene, grosse Makrosporen findet, die weit von der Epidermis getrennt sind. Einzelne Epidermiszellen können sich tangential teilen, aber es ist klar, dass das »Versenken» der Makrosporen nicht durch die Bildung einer Epidermiskalotte erfolgte.

Die Makrosporen liegen aber oft nicht linear, die »Tetrade» hatte nämlich sehr verschiedenes Aussehen. Die EMZ und die beiden Dyadenzellen wurden offenbar häufig durch schiefe oder longitudinale Wände geteilt; es ist wohl die wechselnde Form der EMZ, die diese Variation teilweise bedingt. Sonst seltene Anordnungen der Makrosporen beobachtet man hier oft. Die Tetrade in Fig. 1 zeigt eine beinahe »tetraëdrische» Anordnung der Makrosporen. Die »quadratische» Anordnung mit genau übereinander liegenden Sporenpaaren wurde auch beobachtet. Recht häufig scheint eine Querteilung der mikropylaren und eine longitudinale oder schräge Teilung der chalazalen Dyadenzelle zu geschehen (Fig. 6 u. 31). Diejenigen Makrosporen, die nicht sehr schnell degenerieren, persistieren umgekehrt lange: die Gametophytenentwicklung bleibt eine Zeit stationär, während der Nuzellus wächst, und die Integumente aufwachsen.

Der ES wird hier nicht von einer bestimmten Makrospore gebildet. Oft schien sich die chalazale (vierte) zu entwickeln (Fig. 32), oft war es aber die dritte (Fig. 6), und Fig. 2 zeigt eine der mikropylaren Makrosporen als zweikernigen ES, während zwei andere sich zwar etwas vergrössert haben, aber einkernig sind. Die potentielle Entwicklungsfähigkeit aller Makrosporen äussert sich auch in der Bildung mehrerer ES-e, die recht häufig geschah. Fig. 4 zeigt einen grossen vierkernigen, einen zweikernigen und einen einkernigen ES. Unten sollen weitere Beispiele erwähnt werden. Ich finde keine Veranlassung zur Annahme, dass wie bei *P. pratensis* aposporische ES-e vorkommen. Die neben dem grossen ES in Fig. 3 liegende zweikernige Zelle ist also eine Makrospore, nicht eine Nuzelluszelle. Es wurde übrigens beobachtet, dass

bei *P. alpina* die Kerne diploider ES-e sehr oft zwei Nukleolen haben; wenn nur einen, ist dieser sehr gross. Nach Figuren zu beurteilen, ist die Nukleolarsubstanz auch bei *P. pratensis* in aposporischen, diploiden

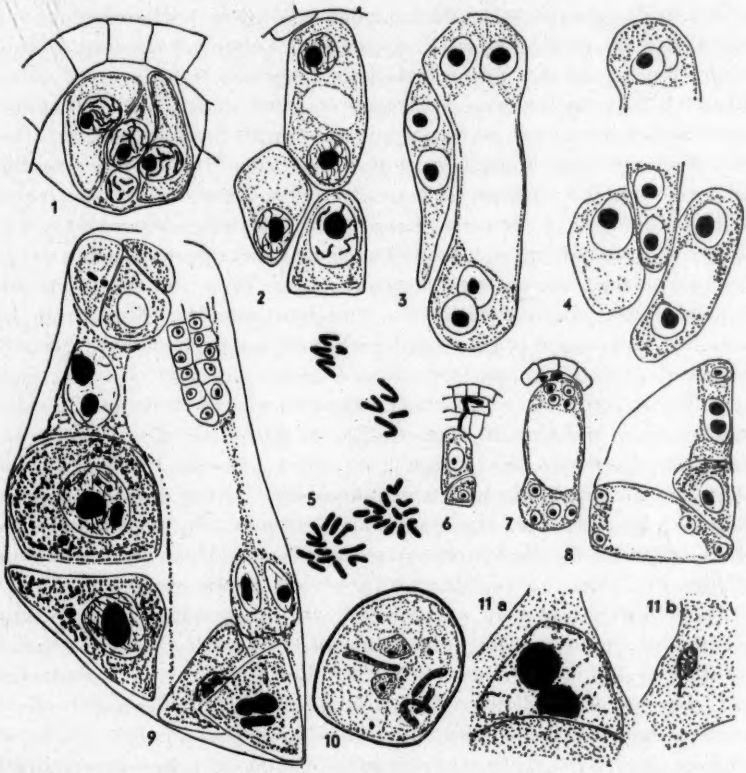

Fig. 1—11. Die Entwicklung bei sexuellen Sippen von *Poa alpina*. — Fig. 1, Makrosporentetrade (91). × 725. — Fig. 2, zwei Makrosporen und zweikerniger ES (57). × 725. — Fig. 3, zweikerniger und vierkerniger ES (91). × 725. — Fig. 4, die Keimung von drei Makrosporen (91). × 725. — Fig. 5, die Kernteilungen eines zweikernigen ES (45). × 1500. — Fig. 6, die mikropylaren Makrosporen sind degeneriert (57). × 280. — Fig. 7, junger achtkerniger ES (45). × 280. — Fig. 8, vierkerniger und normaler ES. Vom letzteren sind Eizelle, Zentralkern und eine Antipode im Schnitte (111). × 280. — Fig. 9, unbefruchteter und befruchteter (?) ES aus einer älteren Samenanlage, letzterer hat abnormen Embryo und wenige Endospermkerne (111). Der Figur ist aus zwei Schnitten kombiniert. × 280. — Fig. 10, abnormes Pollenkorn mit vier Spermakernen und zwei Schlauchkernen (111). × 725. — Fig. 11, Befruchtung des Zentralkerns, *a*: Zentralkern an eine Antipode gedrückt, *b*: Spermakern (90). × 725.

ES-en vermehrt. Dies kann dazu beitragen, aposporische ES-e von haploiden zu unterscheiden.

Die Keimung mehrerer Makrosporen ist ja eine gewöhnliche Erscheinung, wenn der Nuzellus ein s. g. mehrzelliges Archespor hat, und zahlreiche Makrosporen gebildet werden. Bei dem Vorhandensein von nur einer EMZ ist sie selten. Dass sie bei *P. alpina* vorkommt, beruht wohl teilweise auf der unregelmässigen Lage der Makrosporen, die es mit sich bringt, dass mehrere Makrosporen gleich günstig zum Nahrungsstrom in der Samenanlage liegen können (vergl. SCHNARF, 1929). Insbesondere muss die Längsteilung der chalazalen Dyadenzelle die Bildung von zwei ES-en begünstigen. Bei einem *Salix*-Bastard, *S. cinerea* × *phylicifolia*, teilte sich die chalazale Dyadenzelle ungeachtet seiner Länge oft longitudinal, sodass die chalazalen Makrosporen genau neben einander lagen, sie entwickelten sich gern beide (unveröffentlichte Beobachtung). Dies bestätigt die Annahme von der Bedeutung der Lage der Makrosporen als Keimungsfaktor; andere *Salix*-Bastarde bilden nicht extra ES-e aus.

Die Frequenz von extra ES-en war in den verschiedenen Pflanzen verschieden. Meistens ist nur ein ES im Nuzellus. Die Grösse, insbesondere die Breite, des jungen ES variiert. Er ist ohne Zweifel haploid: die in Fig. 1 abgebildeten Makrosporen hatten in ihren Kernen etwa 12 Chromosomen, Fig. 5 zeigt die Kernteilungen des zweikernigen ES von Pflanze 45, die Chromosomenzahl ist 12 (die unreduzierte Zahl ist hier 25). Der ES verdrängt viele sterile Zellen im Nuzellus, doch nicht die Epidermiszellen am Scheitel, die somit den ES vom Mikropylenkanal trennen. Die Antipoden werden bald durch Mitose zweikernig (vergl. Fig. 34), bisweilen wird ihre Kernzahl noch vermehrt, und offenbar können diese Kernteilungen von Zellteilungen begleitet werden, denn bisweilen sieht man 4—5 Antipoden. In älteren ES-en wachsen in der Regel die Antipoden bedeutend, ihre Kerne verschmelzen aber wieder zu einem grossen Kern, der verschiedene Veränderungen erleidet. Das Aussehen älterer Antipoden ist charakteristisch, sie sind breit birnenförmig (wie in Fig. 33 in ÅKERBERG, 1942). Sie persistieren lange nach der Befruchtung. Die Polkerne kommen bald in Kontakt. Oft verschmelzen sie zu einem Zentralkern mit zwei grossen Nukleolen (Fig. 11), oft unterbleibt die Verschmelzung. Sie nehmen bedeutend an Grösse zu. Die Synergiden zeigen bisweilen die speziellen Strukturen, die oft beschrieben sind: ihre Spitzen können gekrümmt sein und eine filare Struktur zeigen, und eine sog. Leiste kann beobachtet werden. Letztere schien durch Schrumpfung ent-

standen. Der Eikern hat einen Nucleolus. Die Zellen des Eiapparates wachsen etwas vor der Befruchtung. Der ES wird allmählich recht stark gekrümmt, die Chalazaregion kommt dadurch scheinbar näher dem Eiapparat.

Spuren der Befruchtung wurden in allen Pflanzen gesehen, in 90 wurden Spermakerne im ES beobachtet. Die reifen Pollenkörner haben nackte Spermakerne, die sehr langgestreckt sind, oft sind sie schwach halbmondförmig gekrümmt und haben zugespitzte Enden. Sie haben eine deutliche Netzstruktur (Wabenstruktur) und es fehlt ihnen ein Nucleolus (Fig. 10). Eine Plasmahülle konnte nicht beobachtet werden. Das abgebildete Pollenkorn ist abnorm, es hat zwei degenerierende Schlauchkerne und vier Spermakerne. Es wurde in Pflanze 111 gefunden. Vermutlich blieb die Wandbildung nach der zweiten Kernteilung einmal aus, und die Dyadenzelle bildete ein Pollenkorn mit zwei Kernen. Es war bedeutend grösser als die normalen Körner. Im ES haben die Spermakerne nicht die langgestreckte Form. Fig. 11 *a* u. *b* zeigen einen Spermakern neben dem Zentralkern, der an eine Antipode gedrückt war, und also seinen ursprünglichen Platz verlassen hat. Am Scheitel der Antipodengruppe geschieht wahrscheinlich die erste Kernteilung des Endosperms, denn im zweikernigen Endosperm lag der eine Kern ganz oberhalb, der andere unterhalb der Antipoden. Vermutlich wandert der Zentralkern mit dem Spermakern zu den Antipoden und die beiden Kerne bilden eine gemeinsame Kernspindel. Die Entwicklung des Endosperms beginnt früher als die Embryobildung. Die Kernteilungen sind anfangs ziemlich simultan, man kann recht häufig »Elimination» von einem Stück eines Chromosoms in den Anaphasen beobachten.

Der Pollenschlauch wurde in dem Mikropylenkanal beobachtet, sein Ende zwischen Synergide und Embryo. Eine Synergide wird offenbar verwendet, nach der Befruchtung ist ihr Kern geschrumpft und sie hat einige chromophile Reste, die Synergide wird aber nicht so stark umgewandelt, wie es bei vielen anderen Angiospermen der Fall ist. Chromophile Substanz ist längs den Zellen des Eiapparates nach der Befruchtung zu sehen. Der Spermakern wurde bei oder in der Eizelle beobachtet, seine Struktur war aber hier undeutlich. Nach der Kernverschmelzung hat der Eikern immer zwei Nukleolen von verschiedener Grösse, der kleinere ist ohne Zweifel vom Spermakern gebildet. Bei *Crepis capillaris* ist wie bei anderen Objekten deutlich festgestellt, dass der Spermakern, der selbst keinen Nucleolus hat, einen solchen nach seinem Eindringen in den Eikern ausbildet (GERASSIMOWA,

1934). Die Teilung der Eizelle geschieht erst nachdem eine Anzahl Endospermkerne gebildet sind. Vor der Teilung ist die Eizelle gewachsen. Die Teilung wurde beobachtet und zeigte die diploide Chromosomenzahl. Die Embryobildung von *Poa annua* ist von SOUÈGES genau untersucht und beschrieben worden (1924).

Wenn sich mehrere ES-e entwickeln, können komplizierte und bisweilen schwer zu deutende Verhältnisse herrschen. Es sollen hier einige solche beschrieben werden. In 45 gab es neben dem achtkernigen ES zwei grosse Zellen, offenbar persistierende Makrosporen. Eine andere Samenanlage zeigte zwei vollständige ES-e. In 91 war die Keimung mehrerer Makrosporen recht häufig, es wurde einmal eine Samenanlage mit zwei Paaren von Polkernen und mehr als acht Antipoden beobachtet, die Verhältnisse nahe dem Mikropylenkanal waren nicht so deutlich. Mehrmals wurden zwei vollständige ES-e im Nuzellus beobachtet. In 111 waren aber extra ES-e am häufigsten, sie konnten recht verschiedene Lage und Ausbildung zeigen, Fig. 8 zeigt einen quergestellten vierkernigen ES unter dem normalen. In einigen Fällen hatten alle vier Makrosporen sich weiterentwickelt, so hatte ein Nuzellus einen fertigorganisierten, breiten ES, einen fertigorganisierten, schmalen ES mit unvollständigem Eiapparat, eine zweikernige Makrospore und einen abnormen ES mit vier Kernen, die in einer Reihe neben einander lagen. Ein grosser Nuzellus hatte zwei grosse ES-e, von denen der eine abnorm ausgebildet war und nicht analysiert werden konnte, und zwischen ihnen war ein sehr kleiner, quergestellter, zweikerniger ES. Der grosse vollständige ES war auch quergestellt und lag nahe der Chalaza.

Es wurde kein Beispiel dafür gefunden, dass mehr als ein ES befruchtet war; das untersuchte Material ist doch nicht gross. Eine Samenanlage hatte ein ziemlich grosses Endosperm, einen vierzelligen Embryo und ausserdem einen ES mit grossem Zentralkern, grossen Antipoden und undeutlichem Eiapparat. Auch betreffs so alter Stadien zeigte 111 die auffallendsten Störungen. Fig. 9 zeigt die beiden ES-e von einer Samenanlage. Der eine ES ist normal entwickelt, seine Eizelle ist offenbar unbefruchtet. Der andere hatte einige grosse Kerne, es sind Endospermkerne, die aber statt 2—3 nur einen Nucleolus haben, Synergiden fehlten, an der Stelle des Eiapparates war ein abnorm entwickelter Embryo. Der Embryo war nämlich überall gleich dick und seine Kerne hatten alle nur einen Nucleolus. Ob eine Befruchtung hier geschehen ist, kann nicht festgestellt werden. Eine Samenanlage hatte einen ES mit Eizelle und grossen Polkernen und einen zweiten mit vier

grossen wandständigen Endospermkernen mit einem Nucleolus und einem vielzelligen (etwa 40 Zellen) Embryo. Im Verhältnis zu der Grösse und dem Alter der Samenanlage schien der Embryo zu gross, um durch eine normale Befruchtung gebildet zu sein. Der Embryo lag neben der Eizelle. Eine Samenanlage hatte einen alten unbefruchteten ES mit sehr grossem Zentralkern, ausserdem ein kleinzelliges Endosperm (Embryo?) und einzelne Antipoden, die einem anderen ES angehörten. Das Endosperm schien nicht weiter entwicklungsfähig zu sein. Schliesslich hatte eine Samenanlage von 111 einen einzigen ES, der vermutlich diploid war. Der Eikern hatte nämlich zwei Nukleolen, jeder Polkern zwei grosse Nukleolen, die Antipoden waren schwach entwickelt.

DIE APOMIKTISCHEN BIOTYPEN.

Es wurden zwei Pflanzen untersucht: Nr. 5 war von einem Biotypus aus Korpilombolo, Nordschweden, 35—4—21 gehörte einem Biotypus aus Gotland an. Im folgenden werden sie nur K. und G. genannt. Die K.-Apomikte hat nach MÜNTZING 38 Chromosomen, der untersuchte Klon war als Vater in der Kreuzung Sexuell × Apomikt verwendet. G. hat 33 Chromosomen, aber diese Rasse hatte auch Individuen mit etwa 49 (triploid) und 66 (tetraploid) Chromosomen (siehe Tabelle 1 in MÜNTZING, 1940). Diese Rasse ist morphologisch sehr frappant. 35—4—21 war eine diploide Pflanze. Die Chromosomenpaarung in der PMZ ist bei K. verhältnismässig gut, in Metaphase 1 kommen 0—5 Univalente vor (MÜNTZING, 1940). Die G.-Apomikte hat MÜNTZING nicht untersucht. Die Diakinese der PMZ zeigt nur einzelne Univalente, näher habe ich sie aber nicht studiert; die Paarung ist also auch hier gut. Die Pollenkörner sind normal ausgebildet (Fig. 26). Pseudogamie ist in *P. alpina* nicht experimentell erwiesen, was auf der geringen Grösse der Blüten beruht. Sie wurde aber als wahrscheinlich betrachtet. Von Professor MÜNTZING bekam ich seine Präparate über frühe Stadien in der Entwicklung der Pajala-Apomikte zur Untersuchung.

Die ganz junge Samenanlage hat einige grosse subepidermale Zellen (Fig. 12), die seitlichen von ihnen werden aber durch perikline Wände geteilt, nur die axile wächst zu einer Archesporzelle aus. Sie bekommt allmählich eine beträchtliche Grösse, und ihr Kern teilt sich viel später als bei den Sexuellen, nämlich erst wenn die Antheren Pollentetraden oder junge Pollenkörner haben. Der Nuzellus hat nun an Grösse zugenommen, und das innere Integument ist über seinem Scheitel emporgewachsen, der Entwicklungsgrad der Blüte ist also eher

wie bei der ersten Kernteilung im ES der Sexuellen. Der Kern der EMZ wächst sehr, er wandert von dem mikropylaren Teil der EMZ gegen ihre Mitte und bekommt eine langgestreckte, bisweilen unregelmässige Form. Letztere wird durch grosse Vakuolen verursacht, die im Zytoplasma der EMZ auftreten und den Kern deformieren. Die Verspätung der Teilung und das Auftreten von Vakuolen sind bei vielen Apomikten mit diplosporischer Entwicklung nach dem *Antennaria*-Schema beobachtet worden, und die folgende Kernteilung hat dann mitotischen Charakter. Die EMZ verwandelt sich in einen einkernigen ES. Der ein- oder zweikernige ES hatte eine etwas verschiedene Form. Bei der Pajala-Apomikte ist er in seinem chalazalen Teil erweitert (siehe Fig. 18 u. 19 in MÜNTZING, 1940), bei G. fehlt diese Erweiterung (Fig. 14), bei K. ähnelt er mehr dem ES der Pajala-Apomikte.

Es wurde nicht beabsichtigt, die Kernteilung näher zu studieren. ÅKERBERG bildet eine Anaphase ab und findet, dass »the first division appears to be quite mitotic» (l. c. S. 93). Die Prophase wurde bei den drei Apomikten gesehen (Fig. 13). Der Kern ist grösser als aus der Figur hervorgeht. Er hatte nur ungepaarte Chromosomen, einfache, oft umgebogene Fäden, die meistens peripher an der Kernmembran lagen. Bei K. wurden etwa 38 gezählt. Es scheint aber die Regel zu sein, dass Diplosporen mit *Antennaria*-Schema mehr oder weniger häufig Meiosis in der EMZ zeigen und Makrosporen bilden, die wohl meistens degenerieren (vergl. BERGMAN, 1941). KIELLANDER fand dies bei den von ihm untersuchten diplosporischen *Poa*-Arten, ja, er fand bei einer Rasse von *P. palustris*, dass etwa 50 % der EMZ Meiosis haben müssen. Bei *P. alpina* wurde aber nur ein ziemlich unsicherer Fall von Makrosporenbildung gefunden. Das untersuchte Material ist nicht gross, aber besonders von G. nicht unbedeutend. Wenn Meiosis vorkommt, muss sie also sehr selten sein. MÜNTZING hat aber eine Apomikte aus der Schweiz gefunden, die partiell sexuell war. Hier kommt offenbar Meiosis oft vor.

Der zweikernige ES der Pajala-Apomikte hat einen schmalen mikropylaren und einen erweiterten chalazalen Teil, die Kerne haben meistens zwei, seltener einen sehr grossen Nucleolus. Bei G. hat der ES eine mehr gewöhnliche Form (Fig. 14). Seine Vakuole ist hier sehr gross, einmal wurde beobachtet, dass sie durch den ganzen ES ging und ihn teilte. Die weitere Entwicklung geschieht nun rasch. Die Bildung des achtkernigen ES in K. zeigt Fig. 15. Wie an anderen Objekten festgestellt worden ist, beginnen die Teilungen im chalazalen

Ende etwas früher als im mikropylaren. Mehr als ein ES wurde nie im Nuzellus beobachtet. Er wird erweitert, bisweilen wird er abnorm breit. Er verdrängt angrenzende sterile Zellen. Bei G. wurde dabei Folgendes beobachtet: bisweilen persistierte der Kern einer aufgelösten Nuzelluszelle und konnte eine knäuelartige Struktur haben, z. B. wenn er in Prophase war. Ein solcher Kern lag nun einige Male zwischen dem Eiapparat und dem Zytoplasma des ES, und ich glaubte anfangs, er sei ein Spermakern. Spermakerne treten aber erst spät im ES auf.

Die Antipoden werden auch hier zweikernig, bisweilen kommen weitere Teilungen vor, etwa 16 Kerne wurden in einer Antipode beobachtet. Die Kerne verschmelzen später, aus den vielen Kernen wird einen Kern mit vielen gleichgrossen Nukleolen gebildet. K. hatte meistens drei Antipoden, G. hat oft eine oder zwei sehr kleine von den drei grossen umgeben, kann aber bis acht Antipoden zeigen. In K. wurden, wie übrigens auch bei einigen Sexuellen, Hunderte von extranukleären Nukleolen (?) in einer der Antipoden beobachtet. Der Kern schien in solchen Antipoden immer in Degeneration zu sein (vergl. Fig. 24 und 38). Die Polkerne verhalten sich vielleicht verschieden. In K. wurde anscheinend oft ein Zentralkern mit 2—4 Nukleolen gebildet. Eine wahre Vereinigung schien aber nicht so häufig vorzukommen. In G. war beinahe immer die Grenze zwischen den Polkernen deutlich. Hier hatte auffallend oft der eine Polkern zwei, der andere einen Nucleolus. Einige Male wurden drei Polkerne in Kontakt beobachtet (siehe Fig. 30); einer von diesen ES-en hatte nur zwei Antipoden, so dass es deutlich ist, dass der eine Polkern ein Kern ist, der normalerweise in eine Antipode eingeht. In den anderen Fällen konnte die Herkunft des dritten Polkerns nicht ermittelt werden. Polkerne oder Zentralkern wachsen sehr stark vor der Befruchtung, sie sind bedeutend grösser als bei den Sexuellen, bei ausgebliebener Befruchtung wachsen sie noch mehr. Die Synergiden können wie bei den Sexuellen ausgebildet sein. Bisweilen sind sie von einander verschieden, und die eine war bisweilen einer Eizelle ziemlich ähnlich (Fig. 16). Die Eizelle ist häufig gross, ihr Kern hat oft zwei Nukleolen. Der ES krümmt sich auch hier stark, die Antipodengruppe kommt nahe an den Eiapparat. Einige Male wurden umgekehrte ES-e ausgebildet, ihre Antipoden lagen bei der inneren Mündung des Mikropylenkanals, Eiapparat und Polkerne lagen nahe bei den Antipoden aber weiter unten im ES. Einige Male hatten in noch recht jungen ES-en die Antipoden eine ungewöhnlich starke Entwicklung bekommen, sodass sie den ganzen Raum des ES ausfüllten. Ein ES mit übergrossen Antipoden ist in Fig. 24 abgebildet.

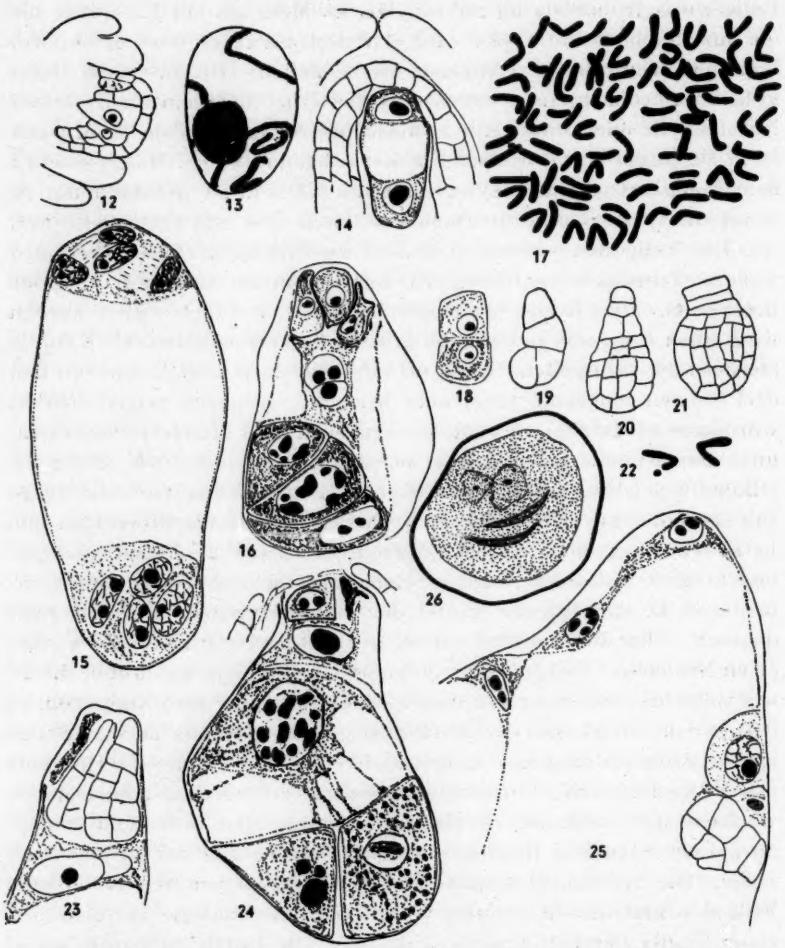

Fig. 12—26. Die Entwicklung bei apomiktischen Biotypen von *Poa alpina*. — Fig. 12, junge Samenanlage mit Archesporzelle (Pajala). × 280. — Fig. 13, ein Teil des Kerns einer EMZ, der mitotische Prophase zeigt (K.) × 2500. — Fig. 14, zweikerniger ES im Nuzellus (G.). × 280. — Fig. 15, der achtkernige ES ist soeben gebildet (K.). × 725. — Fig. 16, der fertige ES (G.). × 280. — Fig. 17, pentaploide Kernplatte mit 81 Chromosomen aus dem Endosperm (G.). × 2600. — Fig. 18, zweizelliger Embryo (K.). × 280. — Fig. 19, vierzelliger Embryo (K.). × 280. — Fig. 20, junger Embryo mit Dermatogen (K.) × 280. — Fig. 21, abnormer Embryo, der sich in dem antimikropylaren Ende eines jungen Samens entwickelt hatte (G.). × 280. — Fig. 22, zwei SAT-Chromosomen aus einer Kernteilung im Embryo. × 2600. — Fig. 23, zwei Embryonen und eine Synergide aus einem unbefruchteten ES (G.).

Er war in einer Samenanlage ohne Mikropylenkanal, und der ES hatte, wie immer in solchen Fällen, die Epidermiszellen an dem Scheitel des Nuzellus flachgedrückt. Weiter ist in solchen Samenanlagen der ES gern etwas abnorm ausgebildet. Besonders eine Antipode ist hier sehr gross, die Eizelle ist niedrig, der Zentralkern hat nur einen Nucleolus. Eine zweite, bedeutend ältere Samenanlage hatte einen ES von recht ähnlichem Aussehen gehabt, sein mikropylares Ende hatte aber keinen Eiapparat, aber zwei recht grosse Embryonen (je von etwa 40 Zellen) und eine Synergide. Beide diese ES-e waren von der K.-Apomikte.

Die Teilung der Eizelle geschieht oft früh, doch erst nachdem die Antipoden ihre definitive Struktur erhalten haben. Die Eizelle wird vergrössert, ehe sie sich teilt; sie hat oft keine Vakuole. Zuerst wird eine Querwand angelegt (Fig. 18), dann teilt sich die apikale, d. h. die gegen den ES gerichtete, Zelle durch eine Längswand, die basale durch eine Querwand (Fig. 19). Es wurden die Teilungen im Proembryo aber nicht genauer verfolgt. SOUÈGES hat eine Schilderung von der Embryobildung bei *Poa annua* gegeben, und einige der beobachteten Proembryonen zeigten dieselben Entwicklungsstadien, die SOUÈGES abgebildet hat. Der Embryo in Fig. 30 *a* und *b* hat acht Zellen, sie bilden vier Etagen. Die beiden basalen Etagen haben je eine Zelle, die dritte hat zwei, die vierte vier Zellen (»Quadrantenstadium»). Fig. 28 *b* zeigt die weitere Entwicklung eines solchen Embryos. Die zweite Zelle wird offenbar durch eine Längswand geteilt, die Zellen der dritten Etage werden wohl beide längsgeteilt, und der Proembryo wird das »Oktantenstadium» erreichen (drei Zellen der vierten — äussersten — Etage waren in einem angrenzenden Schnitt). In der ersten Entwicklung werden mehrere Embryozellen in der Regel gleichzeitig geteilt, später geht aber diese Simultanität der Kernteilungen verloren. Die Entwicklung geht anfangs schnell, es finden sich selten Embryonen mit 3—7 Zellen. Der Embryo wird oft gekrümmt, es werden bald in den apikalen Etagen schiefe und tangentiale Wände angelegt, wodurch Dermatogenzellen abgeschieden werden (Fig. 20).

Die Embryoentwicklung bei diesen Apomikten *kann* also in ganz normaler Weise vor sich gehen, die erwähnte Simultanität der ersten Teilungen scheint doch mehr ausgeprägt als gewöhnlich zu sein. Nun erfolgten aber die Teilungen nicht streng nach einem bestimmten Schema.

× 280. — Fig. 24, ES mit abnorm grosser Antipode in einer Samenanlage ohne Mikropylenkanal (K.). × 280. — Fig. 25, Embryo und Synergide sind an der Seite des ES befestigt, zwischen ihnen ist ein Spermakern, Endospermkerne (K.). × 280. — Fig. 26, Pollenkorn mit zerfallendem Schlauchkern und Spermakernen (G.). × 825.

Variationen in der Reihenfolge der Teilung der verschiedenen Zellen schienen häufig zu sein. Vermutlich kommt aber auch bei den sexuellen Formen von *P. alpina* eine solche Variation vor. SOUÈGES hat vielleicht die Regelmässigkeit der Teilungen zu scharf betont.

Die Kernteilungen im Embryo waren besonders bei K. recht gut fixiert, die diploide Chromosomenzahl konnte festgestellt werden. Die Kernplatte in Fig. 38 *b* und *c* zeigt 38 Chromosomen; von diesen waren 32 in einem Schnitt. Die Form der Chromosomen war oft deutlich, und es wurden auch Satelliten beobachtet (Fig. 22). Es wurden keine Platten mit abweichender Chromosomenzahl beobachtet. Der Embryo in Fig. 30 fällt aber auf, weil beinahe alle Kerne drei Nukleolen haben; in der Regel haben Embryokerne zwei oder einen, nur einzelne können drei zeigen. Der fragliche Embryo ist vermutlich triploid. Oben wurde ein Fall von Polyembryonie erwähnt, es wurden aber weitere solche von sowohl K. als G. gefunden. In allen Fällen lagen zwei Embryonen genau neben einander im selben ES, und neben ihnen lag eine Synergide. Diese konnte geschrumpft sein (Fig. 23), oder gross sein und grossen Kern haben. Der eine Embryo ist also ohne Zweifel von einer Synergide gebildet, eine der Synergiden hatte ja oft eine abweichende Form. In *P. pratensis* ist Polyembryonie häufig, beruht aber auf dem Vorkommen aposporischer ES-e (ÅKERBERG, KIELLANDER); die meisten Fälle von Polyembryonie beruhen auf dem Vorkommen mehrerer ES-e im selben Nuzellus (WEBBER, 1940). Es ist ausgeschlossen, dass sie in *P. alpina* von überzähligen Eizellen kommen. Solche wurden eigentümlicherweise in *Crepis capillaris* beobachtet, in ES-en, die ausserdem die gewöhnlichen acht Kerne hatten (GERASSIMOWA, 1934).

K. und G. sind beide pseudogam. Das Endosperm wird nach der Befruchtung der Polkerne gebildet; wenn diese ausbleibt, teilen sie sich nicht. Das Verhalten der Spermakerne im ES wurde beobachtet. Die »Doppelbefruchtung» der Angiospermen geschieht so, dass der Pollenschlauch unter Mitwirkung einer der Synergiden, die dabei mehr oder weniger desorganisiert wird, sich leert (er giesst sein Inhalt in die Synergide aus ?). Etwas später finden sich zwischen dem Eiapparat und dem Zytoplasma (Protoplast) des ES die beiden Spermakerne und verschiedene chromophile »Reste» von dem Pollenschlauch oder der Synergide; hier ist in Präparaten oft ein durch Schrumpfung gebildeter Zwischenraum zu sehen. Von diesem dringt der eine Spermakern in die Eizelle hinein und befruchtet den Eikern, der andere dringt in das Zytoplasma hinein und befruchtet den Zentralkern. Während die Spermakerne sich im ES befinden, können sie sehr verschiedene Form-

veränderungen erleiden. Sie sind mehrmals beschrieben worden, ausführlich von GERASSIMOWA.

Die Pollenkörner sind etwa wie bei den Sexuellen ausgebildet, der Schlauchkern macht einen kränklichen Eindruck und zeigt bisweilen Zeichen amitotischen Zerfalls (Fig. 26). Die Spermakerne schienen doch kürzer als bei den Sexuellen zu sein. Die Pollenschläuche gelangten zu dem ES bedeutend später als bei den Sexuellen, im Zimmermaterial am 6.—7. Juni statt am 2.—3. Das Öffnen der Blüten und die Anthese geschah viel später bei den Apomikten. Da es sich um ganz verschiedenes Material in den beiden Fällen handelt, ist ein direkter Vergleich schwierig. Es ist doch recht klar, dass bei den Apomikten die Teilung des Kerns der EMZ spät stattfindet, diese Teilung entspricht aber der ersten Teilung im ES der Sexuellen; die Bildung des fertigen ES ist sehr rasch, aber dann verfliesst eine lange Zeit, bis der Pollenschlauch eindringt. Der ES sieht dann älter aus, als es bei den Sexuellen der Fall ist, besonders kann dies aus der Struktur der Antipoden abgelesen werden. Der Pollenschlauch verhält sich wahrscheinlich wie bei den Sexuellen; man findet nämlich eine umgewandelte Syndergide. Im erwähnten Zwischenraum wurden dann die Spermakerne beobachtet, es drang aber nur der eine Spermakern in den Protoplast ein und befruchtete die Polkerne. Die chromophilen Reste waren in G. in grosser Menge vorhanden.

Fig. 27 zeigt die beiden kugeligen Spermakerne, einer ist in den Protoplast eingedrungen und liegt neben den Polkernen, die vom Messer nur tangiert wurden, einer liegt bei dem Embryo. Zwischen dem Embryo, der hier eine mehr ungewöhnliche Form hat, und einer Synergide ist das Ende eines Pollenschlauches. Es enthält zwei kleine Körper, die vermutlich den zerfallenen Schlauchkern darstellen. Der angrenzende Schnitt war defekt, gerade der ES war weggefallen, eine leider recht häufige Erscheinung in diesem Material. Ob hier eine Synergide nicht verwendet wurde, bleibt ungewiss. Fig. 28 ist von K. Die Polkerne haben sich den Antipoden genähert (vergl. S. 31), der Spermakern liegt an die Kernmembran gepresst. Der funktionslose Spermakern lag auf dem Embryo, er war nicht gleichmässig sondern nur in dem einen Ende kontrahiert. Der Embryo ist oben beschrieben, er hatte drei Kernteilungen, Pro-, Meta- und Anaphase; die Metaphase zeigte 38 Chromosomen, die auf zwei Schnitte verteilt waren. Fig. 29 wiederum ist von G., es ist nur ein Schnitt von drei gezeichnet. Der Spermakern in Kontakt mit dem Polkern ist gewachsen und befindet sich im Anfang der Prophase. Oben ist die frische Synergide, ihr Kern

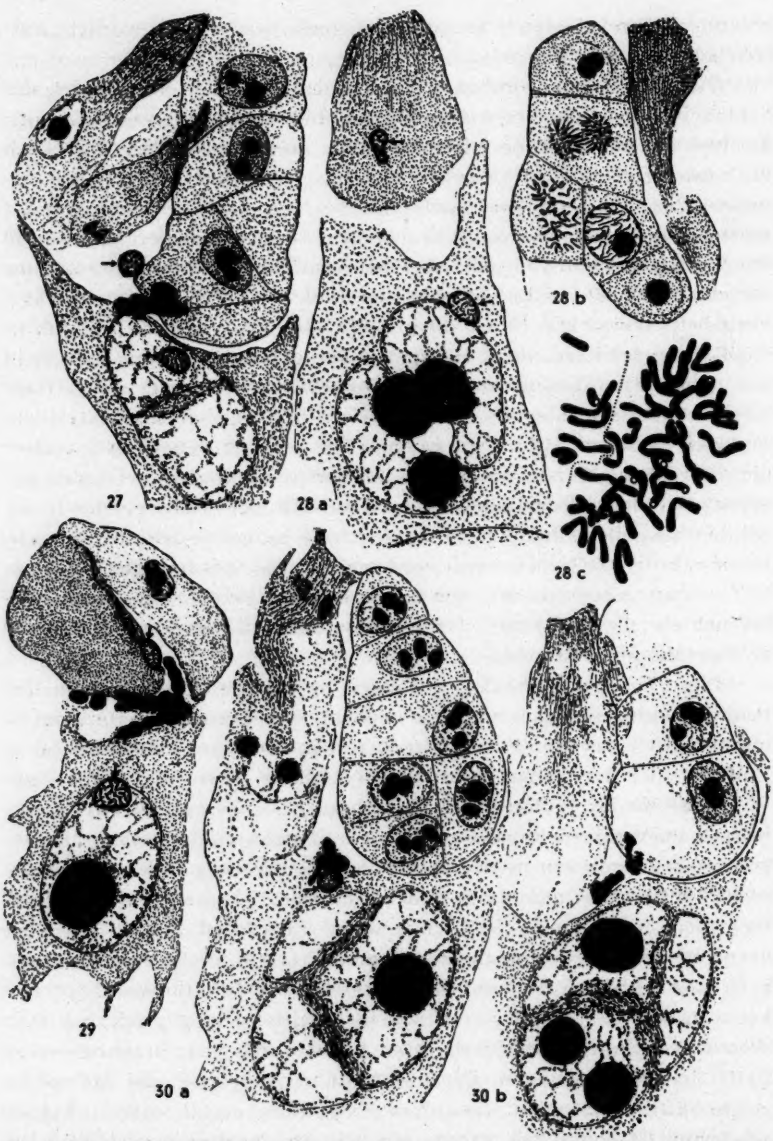

Fig. 27—30. Das Verhalten der Spermakerne im ES der apomiktischen Biotypen von *Poa alpina*. Die Spermakerne sind durch Pfeile ausgezeichnet. — Fig. 27, ein Spermakern ist in den Protoplast eingedrungen, der andere ist neben dem Embryo.

ist nur teilweise in diesem Schnitt; er hatte zwei Nukleolen und deutliche Chromatinfäden. Es ist aber ungewiss, ob man es als Prophase bezeichnen soll, jedenfalls hat die Synergide keine sexuelle Tendenz, da der funktionslose Spermakern offenbar keinen Versuch machte einzudringen. Die chromophilen Reste sind so bedeutend, dass sie kaum mit dem Pollenschlauch hineingelangt sind, es schienen hier neue Ablagerungen hinzuzukommen. Es ist hier vermutlich längere Zeit seit dem Eindringen des Pollenschlauches verflossen, der Embryo war auch grösser, er hatte 23 Zellen. In den anderen Schnitten waren auch die verwendete Synergide, in der kein Kern beobachtet werden konnte, und der Rest eines Pollenschlauches zwischen den oberen Teilen der Synergiden. In Fig. 30 ist der oben erwähnte, vermutlich triploide Embryo abgebildet. In *a* sieht man neben dem Embryo die verwendete Synergide, die oben zwei chromophile Körper hat. Diese Körper können zusammen den desorganisierten Kern der Synergide bilden, was ich am meisten wahrscheinlich finde, weil sie genau in derselben Höhe liegen, und der Kern vermutlich zwei Nukleolen hatte. Man könnte auch an Schlauchkern und Synergidenkern denken. Etwas weiter unten sind die Nukleolen des Kerns der frischen Synergide. Der ES hatte drei Polkerne, und nur ein Spermakern konnte gefunden werden. Es ist wohl möglich, dass hier eine Befruchtung der Eizelle stattgefunden hat, ihre Teilungen müssen aber sehr rasch verlaufen sein.

Die erste Kernteilung des Endosperms wurde hier auch nicht beobachtet, vermutlich bilden die Kerne eine gemeinsame Spindel. Ein Endospermkern kommt oberhalb, der andere unterhalb der Antipodengruppe. Die zweite Kernteilung wurde beobachtet, zwischen Embryo und Antipoden war eine Metaphase, unterhalb der Antipoden eine Telophase. Die Metaphasenplatte hatte 81 Chromosomen (Fig. 17). Da die somatische Chromosomenzahl von 35—4—21 33 ist, so ist also das Endosperm pentaploid, wie man es nach seiner Entstehung aus zwei

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Die Polkerne sind quergeschnitten. Zwischen Synergide und Embryo ist vermutlich das Ende eines Pollenschlauches mit Resten des Schlauchkerns (G.). — Fig. 28, die Polkerne, zwischen denen eine Vakuole sich befindet, die infolge Schrumpfung der Kerne entstanden ist, und ein Spermakern sind hier nahe den Antipoden. Der Embryo ist unvollständig, drei Zellen von der apikalen Etage waren im angrenzenden Schnitt. Die Kernplatte im Embryo hatte 38 Chromosomen (K.). — Fig. 29, der Spermakern, der in Kontakt mit einem Polkern ist, zeigt Prophase, nur ein Teil des Synergidenkerns ist in diesem Schnitt (G.). — Fig. 30, hier wurde nur ein Spermakern entdeckt, die Kerne des Embryos haben aber drei Nukleolen, sie sind vermutlich triploid; es gibt drei Polkerne. In *a* sind die beiden Nukleolen eines Synergidenkerns, und (oben) die bei der Befruchtung verwendete Synergide mit zwei chromophilen Körpern (G.). × 875, Fig. 28 *c* × 2600.

diploiden Polkernen und einem haploiden Spermakern erwarten kann. Die ersten Endospermkerne sind gross, sie haben im allgemeinen mehr Nukleolen als die Endospermkerne der Sexuellen, gern 3—5 statt 2—3. Anfangs sind die Teilungen auch bei den Apomikten simultan, die Entwicklung und Ausbildung des Endosperms ist überhaupt wie bei den Sexuellen.

Wenn Befruchtung nicht eintritt, wird kein Endosperm gebildet. Bisweilen war ein unbefruchteter ES im Ährchen der Feldpflanzen vorhanden, er blieb recht lange bestehen. Ein Vergleich zwischen einigen befruchteten und unbefruchteten ES-en, wobei die letzteren zu den ältesten Blüten der Ährchen gehörten, zeigte folgendes. Der Embryo war etwa ebenso gross in den befruchteten wie in den unbefruchteten, die ersteren hatten aber festes Endospermgewebe, die letzteren hatten Polkerne, die an Grösse stark zugenommen hatten, auch ihre Nukleolen waren grösser, sie waren aber schwächer färbbar als früher. Auch die Antipoden waren verschieden. In den befruchteten ES-en schienen sie eher kleiner als früher, und in diesen hatten sich Vakuolen gebildet, ein Zeichen ihrer Degeneration. In den unbefruchteten ES-en waren die Antipoden enorm gewachsen und hatten noch dichten Inhalt. Die unbefruchteten ES-e hatten an Grösse zugenommen, doch waren sie bedeutend kleiner als die ES-e mit Endosperm. Eine Vergrösserung der Antipoden findet also bei ausgebliebener Endospermbildung statt, vermutlich als eine Folgeerscheinung. Durch die Chalazaregion muss zum ES viel Nahrung kommen, die in diesem Stadium der Entwicklung vom rasch wachsenden Endosperm verbraucht wird. Wenn nun kein Endosperm im ES vorkommt, sollte also die Nahrung von der Antipodengruppe verbraucht werden, die zwischen Chalaza und ES liegt. Die Bedeutung der Antipoden im Angiospermen-ES ist oft diskutiert worden; besonders wenn sie wie bei *Poa* stark entwickelt sind, hat man ihnen eine spezielle physiologische Funktion zugeschrieben. Andere Forscher haben dies aber in Abrede gestellt; die Vergrösserung ist nach ihnen nur eine Hypertrophie, durch den Nahrungsstrom zum ES bedingt. Dies dürfte hier wenigstens von ihrer Entwicklung bei ausgebliebener Befruchtung gelten. Einmal wurde ein ES gefunden, der nur aus Antipodengruppe und Embryo bestand; es war ein abnorm kleiner ES aus der obersten Blüte eines Ährchens. Ob die Polkerne von den Antipoden verdrängt wurden, oder ob der ES von Anfang an unvollständig war, ist unbekannt. In den Zimmerpflanzen blieb die Befruchtung viel öfter aus.

Die Grenzschicht zwischen den beiden Polkernen erscheint in G.

eigentümlich verdickt, wie gestreift (Fig. 30). In alternden Polkernpaaren von K. entsteht oft etwas wie eine Vakuole zwischen den Kernen infolge des Auseinanderweichens ihrer Membranen. Anfangs sieht sie wie in Fig. 28 aus, später wird sie grösser, und die Polkerne sehen dann bei oberflächlicher Beobachtung wie ein einziger ringförmiger Kern mit zwei oder drei Nukleolen aus. Die Bildung dieser Vakuole beruht vermutlich auf Austrocknung, die Kerne verlieren Wasser. In der Regel wird also kein Endosperm gebildet, wenn die Befruchtung der Polkerne ausbleibt. Es fragt sich, ob dies doch nicht ausnahmsweise geschehen kann. In sowohl K. als G. wurden die beiden Polkerne in Prophase beobachtet, trotzdem allem Anschein nach der ES nicht befruchtet war. Dies war in ES-en, in denen der Abstand zwischen Eiapparat und Antipodengruppe so kurz war, dass die Polkerne sowohl den Embryo als eine Antipode berührten. Der Embryo hatte jedesmal etwa 20 Zellen, und es schien ein normaler Zeitpunkt für die Befruchtung zu sein. Eine andere Veränderung hatten die Polkerne in einigen anderen ES-en erlitten, die unbefruchtet waren. Der Abstand zwischen Eiapparat und Antipodengruppe war hier grösser, aber die Polkerne, die ringförmig waren, hatten ihren Platz in der Nähe vom Eiapparat verlassen und lagen am Scheitel der Antipodengruppe, gerade dort wo die erste Teilung des Endosperms stattfinden dürfte. Eine solche Wanderung, die in befruchteten ES-en die Regel sein dürfte, hat also hier stattgefunden, trotzdem sicher kein Pollenschlauch eingedrungen war. Dies sind also die Veränderungen, die an unbefruchteten Polkernen beobachtet wurden, aber ihre Teilung wurde nicht festgestellt. Diese könnte durch das Auffinden tetraploider Kernplatten bewiesen werden, was sich aber als sehr mühsam erweisen würde. NOACK hat in der Kreuzung *Hypericum perforatum* × *quadrangulum* in drei Fällen, also sehr selten, die Bildung von Endosperm vom unbefruchteten Zentralkern in dieser Weise festgestellt. In *P. alpina* ist sie auch, wenn sie überhaupt vorkommt, sehr selten.

Es wurde kein ES angetroffen, der Spermakerne und eine ungeteilte Eizelle hatte. Von der G.-Apomikte fand MÜNTZING 11 Pflanzen mit 33 Chromosomen, 1 mit 41, 1 mit 49, 1 mit 66 und 1 mit 67 Chromosomen, also zwei tetraploide, eine triploide Pflanze und eine mit mehr intermediärer Zahl. Die Tetraploidie könnte durch die Bildung eines tetraploiden ES-es oder durch Befruchtung durch einen diploiden Spermakern erklärt werden, die Triploidie durch Befruchtung durch einen gewöhnlichen Spermakern. Sichere Beobachtungen über ihre Bildung wurden aber nicht gemacht (vergleiche doch den ES in Fig. 30).

Das Zimmermaterial war nicht günstig um frühe Befruchtung zu erhalten. Der funktionslose Spermakern wurde einige Male später bei der Endospermbildung beobachtet. Er war etwas geschrumpft, sein Inhalt schien aber klarer, wie durchsichtig. Einmal hatten beide Spermakerne dieses Aussehen und lagen im erwähnten Zwischenraum. Die Polkerne dieses ES sahen alt aus, und es war offenbar, dass die Spermakerne in Degeneration waren und die Befruchtung ausbleiben würde.

Die weitere Entwicklung des Embryos wurde nicht genau verfolgt. Die Zellteilungen führen lange nicht zu einer Vergrösserung des Embryos, der anfangs (bis vielleicht 50 Zellen) wenig grösser als die ursprüngliche Eizelle ist. Die Embryonen bekommen somit kleine Zellen. Variationen in der Zellteilungsfolge kommen ohne Zweifel vor, denn das Aussehen des jungen Embryos kann wechseln, kurze breite und lange schmale Embryonen wurden beobachtet, jene waren die zellenreicheren. Gegen 100 Zellen kann vermutlich der Embryo ohne Endosperm bekommen. Eine Synergide ist oft lange deutlich und hat einen grossen Kern.

Anomalien kommen vor. Der ES konnte auffallend klein sein. Wenn die Integumente gehemmt waren, lag der Eiapparat höher als normal, weil die Epidermis des Nuzellusscheitels teilweise verdrängt war; ihre äussere Cuticula wurde doch nicht durchbrochen. Fig. 25 zeigt einen Embryo, der nicht bei der inneren Mündung des Mikropylenkanals, sondern seitlich befestigt ist. An seiner Seite ist eine breite Zelle, sicher eine Synergide, deren abweichende Form dadurch verursacht ist, dass sie nicht aus einem schmalen Teil des ES herausgeschnitten wurde (man vergleiche die Synergiden der seitlichen Eiapparate in tetrapolaren ES-en). Der Synergidenkern ist wie häufig kräftig entwickelt. Zwischen Synergide und Embryo ist ein Spermakern, im Zytoplasma sind zahlreiche wandständige Endospermkerne, die Antipoden waren an ihrem gewöhnlichen Platz. Andere Fälle von seitlich befestigten Embryonen wurden beobachtet, so z. B. einer in einem jungen Samen mit festem, von Reservestoffen teilweise erfülltem Endospermgewebe. Dieser Embryo war nicht an dem Mikropylende sondern an dem anderen Ende des Samens befestigt. Der Embryo war ungewöhnlich breit, er war in Suspensor und Embryokugel differenziert, aber der Suspensor bestand nur aus einer Reihe von Zellen, die sehr breit aber auch sehr niedrig waren (Fig. 21).

Die beiden Apomikten zeigten sehr ähnliche Entwicklung. Ich habe aber die Untersuchung einiger Formen angefangen, deren Nachkommenschaft aus sexuell und apomiktisch gebildeten Pflanzen besteht.

DIE F_1-PFLANZEN.

Die Mutterpflanze dieser Kreuzung war von dem hier untersuchten Klon 91, die Vaterpflanze war eine K.-Apomikte. Es wurden fünf Pflanzen von folgenden Klonen untersucht; die Chromosomenzahlen sind von MÜNTZING festgestellt worden.

157	2n = 29	180	2n = 33
178	2n = 33	202	2n = 41

Alle bisher untersuchten Pflanzen aus F_1 und F_2 sind nach MÜNTZING sexuell. Nur 202 ist embryologisch näher studiert worden, aber recht eingehend, es wurden Ährchen von zwei Pflanzen fixiert. 202 ist von recht grossem Interesse, indem 15 % ihrer Nachkommenschaft haploide Pflanzen sind. Die Erzeugung von Haploiden gilt auch für andere F_1-Pflanzen mit hoher Chromosomenzahl; sie haben vom Vater die Tendenz zur parthenogenetischen Entwicklung, von der Mutter zur Bildung reduzierter Gameten (MÜNTZING, 1940). F_1-Pflanzen mit niedriger Chromosomenzahl geben keine Haploiden, MÜNTZING aber vermutet, dass sie gebildet werden, aber zu wenig Chromosomen bekommen, um lebensfähig zu sein. Von 157 und 178 wurden nur jüngere Stadien erhalten, etwas besser wurde 180 untersucht. Alle F_1-Pflanzen bildeten wie die Mutterpflanze in verschiedener Weise orientierte Makrosporen und hatten die Neigung, mehrere ES-e im Nuzellus auszubilden.

In einer neuen Fixierung von 178 konnte die Teilung der EMZ erstmalig studiert werden. Die Metaphase zeigte einige Univalente. Die Kernteilung geschah recht weit unten in der EMZ. Die zweite Teilung wird in Fig. 31 *a* und *b* gezeigt. Verspätete Chromosomen sind in den beiden Kernspindeln vorhanden. Die lange mikropylare Dyadenzelle wird quergeteilt, die kürzere chalazale muss quer- oder längsgeteilt werden (vergl. S. 28). Einmal schien die Teilung des Kerns auszubleiben, in dem Nuzellus war aber eine abnorm grosse somatische Zelle vorhanden.

Zuerst soll die Entwicklung von 202 beschrieben werden. Die Untersuchung einiger beinahe reifen Samen zeigte in einem einen haploiden Embryo; Haploiden werden offenbar recht häufig gebildet. Fig. 32 zeigt eine lineare Makrosporentetrade. Die chalazale Spore bildet hier den ES. Die Keimung mehrerer Makrosporen ist aber eine ebenso häufige Erscheinung wie in der Mutterpflanze, man findet oft zwei voll entwickelte ES-e im Nuzellus. Der eine ES konnte recht ver-

schiedene Lage haben. Seine Antipoden lagen nicht in der Chalaza, bisweilen lagen sie längs der Seite des normalen ES, eine Antipode kann gar neben dem Eiapparat des letzteren liegen. Solche Antipoden haben nicht die gewöhnliche Form, sie sind oft grosse, niedrige Zellen, deren Wand unvollständig sein kann. Die Eizelle des neuen ES kann verschieden plaziert sein. In dem einen ES in Fig. 36 ist sie nicht an der Wand befestigt sondern liegt frei. Neben oder unter den normalen Antipoden war in der Chalaza bisweilen eine grosse, abgerundete Zelle mit einem oder zwei grossen Kernen, vermutlich war es eine Makrospore, sicher ist es aber nicht. Fig. 33 zeigt, dass eine solche Zelle zu einem vierkernigen ES ausgewachsen ist. Die Kerne sind hier kleiner, sie haben zwei Nukleolen. Dies deutet darauf hin, dass der ES diploid ist, und hier sollte also eine Tendenz zur Aposporie vorkommen, die bei den Sexuellen nicht gefunden wurde. Mit Rücksicht auf die hohe Chromosomenzahl hat vielleicht die Nukleolenzahl weniger Beweiskraft; eine haploide Makrospore hat beinahe ebenso viele Chromosomen wie die Nuzelluskerne der Sexuellen, die meist zwei oder einen Nucleolus haben. Eine recht eigentümliche Entwicklung hat der chalazale ES in Fig. 34 erhalten. Der Nuzellus hat zwei ES-e. Der mikropylare ist der grössere, er ist aber unvollständig, seine Kerne liegen nahe der Mitte, es konnten nur drei gefunden werden, vermutlich sind es doch vier. Der chalazale ES ist vollständig mit drei zweikernigen Antipoden, er hat aber eine inverse Lage, sein Eiapparat ist genau gegen die Chalaza gerichtet, er ist an dem gewöhnlichen Platz der Antipoden. Die grosse Zelle hat vielleicht das Aufwachsen in normaler Richtung verhindert. Inverse ES-e sind bisweilen gefunden, sie sind doch sehr selten.

Der fertige ES von 202 hat eine Organisation, die eher an die der G.-Apomikte erinnert als an die Eltern. Die Antipodengruppe hat oft bis acht Zellen, eine oder zwei in der Mitte sind oft klein (Fig. 37). Die Polkerne sind in Kontakt aber deutlich getrennt, drei Polkerne wurden in Kontakt beobachtet, die Synergiden sind oft nicht typisch ausgebildet. Immer hat aber ein Polkern nur einen Nucleolus. Spuren von der Befruchtung wurden mehrmals beobachtet: chromophile Substanz findet sich am Rand der Zellen des Eiapparates, der Eikern hat nach der Befruchtung einen zweiten kleineren Nucleolus. Die Spermakerne wurden seltener beobachtet, sie schienen sehr diffus gefärbt und ohne Strukturen zu sein. GERASSIMOWA (1934) erwähnt aber, dass in *Crepis capillaris* die Spermakerne »in the final phases of their transformation entirely lose their staining capacity». Ein entsprechendes Stadium ist wohl hier beobachtet. Es wurden aber auch Spermakerne vom gewöhn-

lichen Bau im ES gefunden. Die ersten Endospermkerne sind gross, sie haben oft drei Nukleolen. Einige gute Kernteilungen wurden

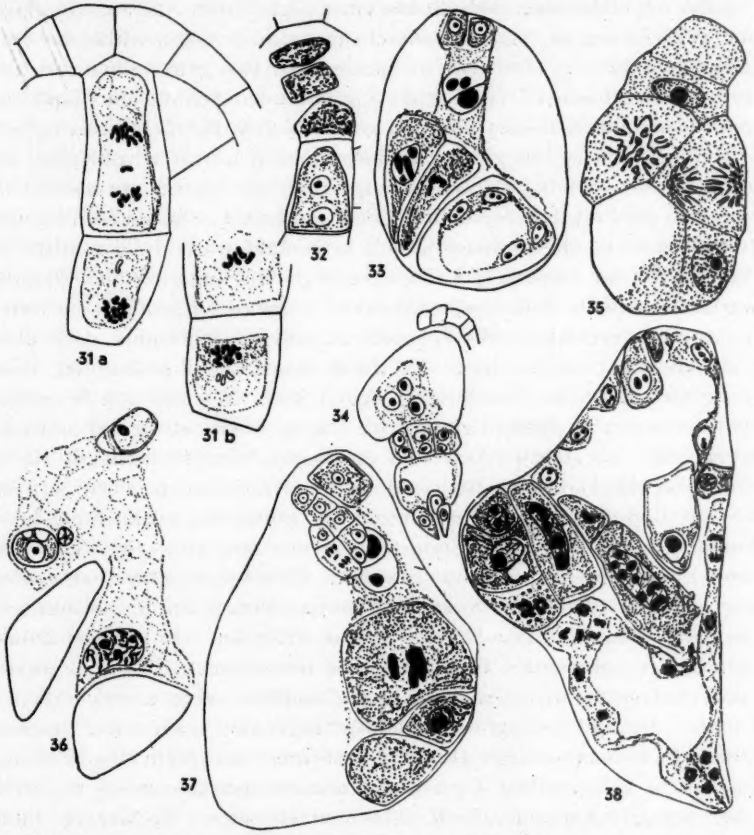

Fig. 31—38. Die Entwicklung von F_1-Pflanzen, Sexuell × Apomikt in *Poa alpina*. — Fig. 31, Anaphase 2, die mikropylare Dyadenzelle wird quer geteilt, die chalazale längs oder schräg geteilt. Elimination von einem bzw. vier Chromosomen (178). × 825. — Fig. 32, eine lineare Makrosporentetrade, schwach entwickelt (202). × 725. — Fig. 33, normaler ES und vierkerniger ES, der letztere aposporisch? (202). × 260. — Fig. 34, ein mikropylarer ES mit drei freien Kernen, ein chalazaler mit Eiapparat, Polkern und zweikernigen Antipoden, der vollständige ES zeigt umgekehrte Polarität, sein Eiapparat ist gegen die Chalaza gerichtet (202). × 280. — Fig. 35, haploider Embryo mit 19 Chromosomen (202). × 875. — Fig. 36, die Samenanlage hatte zwei ES-e, der abgebildete Schnitt zeigt eine freiliegende Eizelle, zwei Spermakerne, einen Endospermkern und zwei Antipoden (202). × 280. — Fig. 37, unbefruchteter ES mit Embryo (202). × 280. — Fig. 38, die Weiterentwicklung von zwei ES-en, Erklärung im Text (180). × 280.

beobachtet, die etwa 60 Chromosomen zeigten. Das Endosperm ist also triploid.

Fig. 36 bildet einen Schnitt aus einer Serie durch eine Samenanlage mit zwei ES-en ab, in die wahrscheinlich zwei Pollenschläuche eingedrungen waren. Oben ist ein Spermakern von gewöhnlicher Struktur. In der gleichen Höhe lag im angrenzenden Schnitt ein Eiapparat mit chromophilen Resten, die den gewöhnlichen Befruchtungsweg anzeigten. Unten ist ein grosser Endospermkern mit drei Nukleolen, im angrenzenden Schnitt war ein zweiter Endospermkern. In einem ES sind also die Polkerne befruchtet, der Eiapparat war zu undeutlich, um zu entscheiden, ob die Eizelle auch befruchtet war. In der Mitte ist die Eizelle des zweiten ES, die frei liegt; im angrenzenden Schnitt waren die grossen Polkerne in Kontakt. Neben der Eizelle ist ein freier Kern, etwas grösser als ein Spermakern und mit Nucleolus, doch aber wahrscheinlich ein solcher. Es wurde sonst nicht beobachtet, dass mehr als ein ES im Nuzellus befruchtet war. Wie bei den Sexuellen schien das Vorhandensein eines extra ES die Entwicklung des befruchteten später zu stören; doch sind die Beobachtungen nicht an einem grösseren Material gemacht. Anfangs ist es nicht so, es wurde oft ein ES mit Endospermkernen und einer mit Polkernen beobachtet, dabei konnten die Polkerne von Endospermkernen umgeben sein. Von späteren Störungen seien folgende erwähnt. Eine Samenanlage hatte einen grossen unbefruchteten ES mit sehr grossen Polkernen, neben ihm war ein Syncytium mit Vakuolen zwischen den Kernen, sehr wahrscheinlich war es das rudimentäre Endosperm des befruchteten ES. Eine zweite Samenanlage hatte einen beim ersten Anblick recht abenteuerlichen Inhalt. Sie hatte ein grosses Endospermgewebe, noch ohne Reservestoffe. In seinem mikropylaren Teil hatte das Endosperm eine Höhlung, in der ein vollständiger ES lag, der ausserordentlich grosse Polkerne aber normale Antipoden hatte. An der Seite dieses ES war ein langgestrecktes Gewebe von kleinen Zellen, offenbar der stark missbildete Embryo des ES mit Endosperm. Die ausgebliebene Vergrösserung der Antipoden des unbefruchteten ES fällt auf. Dies bestätigt die oben erwähnte Auffassung, dass die Vergrösserung bei den Apomikten auf Überschuss von Nahrung infolge ausgebliebener Endospermbildung beruht. In dieser Samenanlage wird wohl die Nahrung vom Endosperm des befruchteten ES verbraucht. In keinem Fall wurden Embryonen in zwei verschiedenen ES-en beobachtet. Einmal hatte eine Samenanlage mit Endospermgewebe zwei Embryonen; aus der Lage ging aber hervor, dass sie wie bei den Apomikten im selben ES gebildet wurden.

Wir haben nun die Frage der Entstehung der haploiden Embryonen zu diskutieren. Wenn man Ährchen von den Sexuellen untersucht, in denen Embryo- und Endospermbildung soeben angefangen haben, findet man eine ungeteilte Eizelle oder einen Embryo von wenigen Zellen aber ein Endosperm mit vielen Kernen. Bei den Apomikten war der Embryo weiter entwickelt als das Endosperm. 202 zeigte aber recht wechselnde Entwicklung, bisweilen wie bei den Apomikten, am öftesten wie bei den Sexuellen; schliesslich konnte der ES ein Endosperm mit wenig Kernen und einen Embryo von wenigen Zellen haben. Eine genauere Untersuchung zeigte, dass die Eizelle sich bisweilen früh teilt, ehe eine Befruchtung stattfinden kann. Fig. 37 zeigt einen ES mit einem Embryo, der 12 Zellen hatte, und zwei Polkerne. Ein ähnlicher ES hatte einen Embryo von etwa 20 Zellen; eine Befruchtung war auch hier ausgeschlossen. Die Kernteilungen in diesen Embryonen waren längsgeschnitten, sie machten aber den Eindruck, dass die Embryonen haploid seien. So wurden Embryonen mit haploiden Kernplatten gefunden. Fig. 35 zeigt einen Embryo, der das Oktantenstadium erreichen soll (einige Zellen waren im angrenzenden Schnitt) und eine Pro-, eine Meta- und eine Anaphase hat. Die Metaphase zeigt 19 Chromosomen, eine Zahl die MÜNTZING in den Nachkommen gefunden hat. Im angrenzenden Schnitt waren zwei Synergiden von recht verschiedener Grösse und ausser diesem ES hatte der Nuzellus eine zweite. Bemerkenswert ist die Simultanität der Teilungen im Embryo, die wir auch bei den Apomikten fanden. In 202 wurden zwei Male 5—6 Prophasen gesehen, d. h. in allen Zellen ausser dem basalen. Später geht diese Simultanität verloren. In durch Befruchtung gebildeten Embryonen dürfte sie nicht so ausgeprägt sein, wurde jedenfalls nicht beobachtet.

Es ist also hier eine Tendenz der Eizelle vorhanden, sich früh zu teilen wie bei den Apomikten, doch ist diese Tendenz nicht so ausgeprägt. Da der ES von einer haploiden Makrospore gebildet ist, sind die frühen Embryonen haploid. MÜNTZING fand ja 15 % Haploide; dies sollte also unter normalen Verhältnissen die Frequenz früher Embryonen sein. Sicher kann aber diese Frequenz unter gewissen Bedingungen, nämlich bei einer Verzögerung der Befruchtung, stark vermehrt werden. Dies ging aus dem Verhalten der Pflanzen in meinem Zimmerfenster vor. Die Antheren öffneten sich bisweilen nicht; von 202 war die Anthese gut in den ersten Infloreszenzen aber sehr schlecht in den späten. Am 14. Juni wurde eine Fixierung gemacht; dies war ein sehr später Zeitpunkt. Höchstens die unterste Samenanlage in jedem Ährchen hatte Endosperm und war somit befruchtet, in jedem

aber hatte wenigstens eine Samenanlage Polkerne und Embryo, in einigen Fällen zeigten Kernteilungen, dass diese Embryonen haploid waren. Man konnte auch Samenanlagen mit völlig degeneriertem Inhalt finden. Auch aus anderen Fixierungen ging es hervor, dass bei dem Ausbleiben von Befruchtung die Eizelle einen Embryo bildet, aber die Polkerne sich nicht teilen, also ganz wie bei den Apomikten. Ohne Befruchtung der Polkerne wird weder Endosperm gebildet noch keimfähige Samen erhalten. Der Entwicklungsfähigkeit der Eizellen ohne Befruchtung ist doch anscheinend gewisse Einschränkungen gesetzt. Es wurde oben hervorgehoben, dass Embryonen nicht in zwei ES-en entwickelt waren. Unmöglich ist es aber vermutlich nicht. Eine Samenanlage mit zwei ES-en hatte eine grosse befruchtete und eine grosse unbefruchtete Eizelle; sie sahen beide teilungsbereit aus und diploiden bzw. haploiden Embryo bilden zu können.

202 ist von MÜNTZING als sexuell angegeben; sie gibt ja eine Nachkommenschaft, die überwiegend aus durch Befruchtung entstandenen Pflanzen besteht. Man kann sie aber auch als eine pseudogame Apomikte bezeichnen, denn die Eizellen der Apomikten sind offenbar befruchtungsfähig. Gemeinsam ist also, dass sich die Eizelle mit oder ohne Befruchtung entwickeln kann, dass aber die Polkerne befruchtet werden müssen. Bei den Apomikten ist die Befruchtung der Eizelle sehr selten, bei 202 geschieht dies unter normalen Bedingungen in 85 % (höchstens) der Samenanlagen. Der Unterschied dürfte bedingt sein durch die durchschnittlich noch frühere Teilungsfähigkeit der Eizelle und noch späteres Eindringen des Pollenschlauches in den ES der Apomikten. Experimentelle Behandlung von 202 kann vermutlich viel häufiger eine Entwicklung ohne Befruchtung erzielen. Eine frühe Teilungsfähigkeit der Eizelle könnte bei einer späten Anthese oder später Empfangsfähigkeit der Narben oder langsamen Zuwachs des Pollenschlauches eine Apomikte mit haploider Nachkommenschaft erzeugen. Eine solche musste in zwei Generationen aussterben. Erst das Erwerben von Diplosporie, Aposporie oder Nuzellarembryonie sicherte ihre Existenz.

Ausser der Teilungsfähigkeit der Eizelle hat 202 bisweilen Züge in der Ausbildung des ES, die an den Apomikten erinnern. Sie wurden doch meistens im Zimmermaterial beobachtet. Die Synergiden hatten eine variierende Ausbildung, ein Synergidenembryo wurde beobachtet, drei Polkerne konnten vorkommen. Die Polkerne verschmelzen nie. Es ist oft eigentümlich schwierig zu konstatieren, ob ein Zentralkern oder zwei Polkerne in intimem Kontakt im ES sind, so z. B. in der K.-

Apomikte und den Sexuellen. In G. und 202 waren die Polkerne aber deutlich getrennt. In den Sexuellen konnte in der Regel keine Grenze zwischen ihnen beobachtet werden, der Zentralkern hatte aber zwei grosse Nukleolen; die ausgebliebene Verschmelzung der Nukleolen beruhte wohl darauf, dass die Vereinigung der Polkerne sehr spät geschah. In K. war es oft erst die bei Austrocknung gebildete Vakuole, die zeigte, dass die beiden Kernmembranen an der Kontaktfläche nicht aufgelöst waren. Wie bei gewissen Sexuellen wird sehr oft in 202 ein zweiter ES ausgebildet. Ich habe ein Ährchen angetroffen, in dem alle Samenanlagen zwei voll entwickelte ES-e hatten, der eine war meistens befruchtet. Es ist schliesslich möglich, dass 202 eine neue Eigenschaft zeigen kann, nämlich die Bildung eines aposporischen ES. Es gab zwar keine Andeutung dazu, dass ein fertigorganisierter diploider ES gebildet wird, es wurde aber einige Male beobachtet, dass neben dem normalen ES ein ES da war, der einige grosse wandständige Kerne mit zwei Nukleolen hatte. Es ist vielleicht so, dass aposporische ES-e nicht zu vollständiger Zellbildung befähigt sind und höchstens formlose Antipoden ausbilden können.

Die F_1-Pflanze 180 hatte eine niedrigere Chromosomenzahl. Auch sie zeigte sehr häufig einen extra ES von recht verschiedener Lage und Organisation. Es wurde nicht beobachtet, dass die Eizelle sich ohne Befruchtung teilte, oder dass der Embryo weiter entwickelt als das Endosperm war. Das untersuchte Material ist aber nicht gross, und es fehlen Fixierungen von älteren Ährchen von Zimmerpflanzen. Es kann also noch nicht behauptet werden, dass ein scharfer Unterschied in bezug auf die Teilungsfähigkeit der Eizelle 202 gegenüber besteht.

Bei 180 wurden zwei Endosperme in derselben Samenanlage beobachtet; es haben sich also zwei ES-e weiterentwickelt (Fig. 38). Die Figur ist aus mehreren Schnitten kombiniert, und Zellen oder Kerne, die nahe einander gezeichnet sind, können voneinander recht entfernt sein; auch ist nicht alles gezeichnet. Der mikropylare ES hatte einige Endospermkerne mit zwei oder drei Nukleolen und eine grosse Eizelle, deren Kern zwei Nukleolen von sehr verschiedener Grösse hatte. Es sind hier Eizelle und Polkerne befruchtet worden. In der Mitte sind die beiden Antipodengruppen. Eine Zelle in der »normalen» Gruppe hatte degenerierten Kern und zahlreiche extranukleare Nukleolen; von den Zellen der zweiten Gruppe sind nur zwei gezeichnet. In der Mitte der Figur sind zwei weitere Zellen; die eine sieht wie eine riesengrosse Eizelle aus, liegt aber frei; die andere ist an der Wand befestigt. Die letztere ist die (unbefruchtete) Eizelle des zweiten ES oder möglicher-

weise eine Synergide, die erstere lag in der Tat von dieser Zelle zwei Schnitte entfernt und kann kaum eine Eizelle sein. Vermutlich ist es eine Makrospore. Der Kern der Eizelle war stark chromophil, ein Zeichen seines Alterns. Unten in der Figur ist das Endosperm des zweiten ES; es hatte recht viele Kerne in Telophase. Dieser ES war mehr in der Querrichtung der Samenanlage entwickelt. Dass es ein anderes Endosperm als das mikropylare war, ging deutlich aus dem Präparat hervor. Die fragliche Samenanlage ist somit von zwei Pollenschläuchen besucht.

ALLGEMEINER TEIL.

Agamospermie — die apomiktische Erzeugung von Samen — kann in verschiedener Weise geschehen. Bei *Poa alpina* teilt sich der Kern der EMZ durch Mitose, die EMZ verwandelt sich direkt zu einem diploiden ES, die Eizelle bildet ohne Befruchtung den Embryo. Nach dem Vorschlag von EDMAN (1931) wird diese Fortpflanzung Diplosporie genannt, und die Entwicklung des ES geht nach dem *Antennaria*-Schema. Man muss aber immer sehr vorsichtig sein bei der Behandlung apomiktischer Vorgänge bei den Angiospermen, denn es kommt hier besonders oft vor, dass verschiedene Forscher demselben Terminus verschiedenen Inhalt geben. GENTCHEFF und GUSTAFSSON (1940 a u. b) verwenden Diplosporie in derselben Meinung wie EDMAN. FAGERLIND (1940) und nach ihm STEBBINS (1941) behalten die Bezeichnung Diplosporie, geben ihr aber eine andere Bedeutung. Sie sprechen von Diplosporie, wenn die Kernteilung in der EMZ einen stark meiotischen Charakter hat, die zu der Bildung eines Restitutionskerns führt. In dieser Meinung ist *P. alpina* nicht diplosporisch. FAGERLIND, STEBBINS und BERGMAN (1941) sollten die Entwicklung in *P. alpina* als generative Aposporie bezeichnen, was nach ROSENBERG (1930) auch gut möglich ist. Es scheint die Regel zu sein, dass bei diplosporischen Formen der Kern sich mehr oder weniger häufig durch Meiosis teilt und haploide Makrosporen gebildet werden (siehe besonders BERGMAN, 1941). Hier wurde dies noch nicht sicher festgestellt, das untersuchte Material ist aber nicht gross. Jedenfalls muss aber Meiosis in den bisher untersuchten Biotypen sehr selten sein.

Die Eizelle hat allem Anschein nach ihre Sexualität nicht verloren, sie kann, wenigstens bei der Gotland-Apomikte, wahrscheinlich befruchtet werden. Alle beobachteten ES-e, in die Pollenschläuche eingedrungen waren, hatten zwar einen Embryo, in einem Fall war aber der

Embryo wahrscheinlich triploid, und der ES hatte nur einen statt zwei freie Spermakerne. Weiter sind von dieser Apomikte triploide Pflanzen von MÜNTZING gefunden worden.

Die Apomikten von *P. alpina* sind pseudogam. Nach GENTCHEFF und GUSTAFSSON (1940 b) sind viele Fälle von Pseudogamie bei Apomikten mit (somatischer) Aposporie bekannt, die Kombination von Diplosporie mit Pseudogamie war aber nicht festgestellt, doch aber in *Poa alpina* und *Zephyranthes texana* wahrscheinlich. Bei *P. alpina* wurde aber durch die embryologische Untersuchung Pseudogamie sichergestellt, und es konnte hier zum ersten Mal das Verhalten der Spermakerne im ES in Formen mit induzierter Apomixis näher studiert werden. Das Verhalten des Pollenschlauches ist hier in Sexuellen und Apomikten ähnlich, und die beiden Spermakerne gelangen in den Raum zwischen dem Eiapparat und dem sog. Protoplast des ES. Bei den Sexuellen dringt dann ein Spermakern in die Eizelle, der andere in den Protoplast und befruchtet die Polkerne. Bei den Apomikten verhalten sich die Spermakerne verschieden, der eine bleibt im erwähnten Zwischenraum und degeneriert, der andere dringt in den Protoplast ein und befruchtet die Polkerne, verhält sich also ganz normal. Dies Verhalten des funktionslosen Spermakerns ist bemerkenswert, man findet ihn bisweilen auf dem Embryo oder zwischen Synergide und Embryo. Recht klar ist es, dass er seine Sexualität nicht verloren hat. Dass er nicht fungiert, beruht darauf, dass die Eizelle sich schon geteilt hat. Wenn der Pollenschlauch vor dieser Teilung in den ES eindringt, findet sicher eine Befruchtung des Eikerns statt, dies dürfte aus den obigen Ausführungen hervorgehen.

Der »funktionslose» Spermakern dringt nicht in den Protoplast des ES hinein, was vielleicht unerwartet ist. Sein Nichteindringen kann möglicherweise darauf beruhen, dass die beiden Spermakerne des Pollenschlauches chemotaktisch durch verschiedene Stoffe angezogen werden, und also Eikern und Polkerne entsprechend verschiedene Stoffe aussondern. Man kann sich aber auch vorstellen, dass die Grenzschicht des Protoplasts bei dem Eindringen des ersten Spermakerns eine Veränderung erleidet, sodass der Eintritt von mehr Kernen verhindert wird. Ein solches Verhalten ist bei tierischen und pflanzlichen Eiern, die sich im Wasser befruchten und entwickeln und so gross sind, dass sie näher studiert werden können, bekannt. Nach dem Eindringen der Spermie wird hier eine Membran ausgebildet, die das Eindringen weiterer Spermien verhindert. Es scheint das Verhalten von überzähligen Spermakernen im ES, das bei verschiedenen Objekten

festgestellt ist, für die letztere Auffassung zu sprechen. Ausserordentlich selten ist nämlich »dispermische» Befruchtung von Eikern oder Polkernen beobachtet worden. Überzählige Spermakerne sind aber nicht so selten (siehe SCHNARF, 1929), bei *Crepis capillaris* kommen sie nach GERASSIMOWA (1934) häufig vor, nur zwei Spermakerne führen aber Befruchtungen aus, die überzähligen bleiben zwischen Eiapparat und Protoplast. Dies deutet meiner Meinung nach stark auf eine Veränderung der Membranen von Eizelle und Protoplast.

Die bekannte Untersuchung von PACE über *Zephyranthes texana* zeigt Verhältnisse, die teilweise an die hier geschilderten erinnern (PACE, 1913). Der Pollen ist normal, und der Pollenschlauch bringt zwei Spermakerne zum ES. Die Verschmelzung des einen mit den Polkernen wurde beobachtet, der andere drang in die Eizelle ein, verschmolz aber nie mit dem Eikern sondern degenerierte, während der Eikern sich unbefruchtet teilte. Die Untersuchung ist an grossem Material ausgeführt und von guten Figuren begleitet, an der Richtigkeit der Beobachtungen kann anscheinend kaum gezweifelt werden. Die Bedeutung dieser Untersuchung wurde von verschiedenen Seiten durch den Hinweis, dass Pseudogamie in *Zephyranthes* nicht festgestellt ist, teilweise in Frage gestellt. Auch wurde die Aufmerksamkeit in erster Linie auf das eigentümliche Verhalten der Eizelle gelenkt. Es schien eine Stimulation der Eizelle durch Eindringen eines Spermakerns vorzuliegen, also Pseudogamie in einer mehr eingeschränkten Bedeutung des Wortes, wie sie bei verschiedenen zoologischen Objekten festgestellt ist. Bei *Rhabditis*-Arten ist aber erwiesen, dass die Spermie, die selbst degeneriert, doch ein Zentrosom dem Ei zuführt, wonach die Teilungen beginnen (BĚLÁŘ, 1924). Eine solche Stimulierung brauchen nicht typisch zentrosomlose Zellen, und das Verhalten der Eizelle in *Zephyranthes* erscheint also nunmehr noch eigentümlicher und verdient eine nochmalige Überprüfung.

Wenn die Befruchtung der Polkerne ausbleibt, was in der Regel darauf beruht, dass kein Pollenschlauch zu der Samenanlage gelangt (einmal wurden aber die beiden Spermakerne in Degeneration im »Zwischenraum» beobachtet, dieser ES war zu alt um den Eintritt des Spermakerns in den Protoplast zu erlauben ?; die Grenzschicht war verändert ?), teilen sich die Polkerne nicht. Nur wenige Male wurde solchenfalls Prophase in den beiden Polkernen oder eine Wanderung der Polkerne zu dem Scheitel der Antipodengruppe als Einleitung zur Endospermbildung beobachtet. Bei ausgebliebener Befruchtung erleidet der ES gewisse Veränderungen, vermutlich eine

Folge davon, dass kein Endosperm vorhanden ist, so dass es momentan einen Überschuss an Nahrung gibt. Der unbefruchtete ES nimmt wie die Samenanlage etwas an Grösse zu, es ist aber klar, dass der Zuwachs von ES und von somatischen Teilen der Samenanlage viel stärker ist, wenn die Befruchtung der Polkerne geschieht und Endosperm gebildet wird.

Die Embryoentwicklung der Apomikten kann völlig normal vor sich gehen. Ohne Endosperm können die Embryonen vielleicht eine Grösse von etwa 100 Zellen erreichen; doch wurden keine Beobachtungen über ihr weiteres Verhalten gemacht.

Die sexuellen Sippen bilden haploide Makrosporen. Es hatten alle Makrosporen eine Tendenz sich zu ES-en zu entwickeln. Teilweise geht dies auf die Form der EMZ zurück, die abweichende Makrosporenanordnungen hervorruft. Die Pflanze 90 hatte in der Regel nur einen ES im Nuzellus, 91 und besonders 111 und die Bastardpflanzen aber sehr häufig zwei. Die Keimung mehrerer Makrosporen ist eine recht gewöhnliche Erscheinung in Pflanzen mit mehreren EMZ-n und Makrosporentetraden, aber eine seltene, wenn nur eine Tetrade vorhanden ist wie in *P. alpina*. Überzählige ES-e konnten abweichende Züge in ihrer Organisation zeigen. Es gibt gewisse doch kleinere Unterschiede zwischen sexuellen und apomiktischen ES-en. Die Kerne der ersteren haben immer nur einen Nucleolus (abgesehen von den Antipodkernen), die Kerne der letzteren haben häufig zwei, vermutlich infolge der Diploidie; besonders häufig hatte der eine Polkern einen, der andere zwei Nukleolen. Sie können, wie bei anderen Apomikten auch festgestellt worden ist, nicht selten abnorme Züge zeigen, die oben beschrieben wurden.

Die Bastarde zwischen Sexuellen und Apomikten sind sexuell. Es wurden einige Pflanzen untersucht, davon 202 eingehend. Sie bilden haploide Makrosporen und wie bei der sexuellen Mutterpflanze werden häufig mehrere ES-e entwickelt. Die Eizelle von 202 kann sich ohne Befruchtung teilen, die Polkerne müssen aber befruchtet werden, um diese haploiden Embryonen zur Reife zu bringen. Unter normalen Verhältnissen hat 202 auch gegen 15 % Haploide in der Nachkommenschaft, bei ausgebliebener Pollination werden weit mehr Embryonen ohne Befruchtung gebildet. Der Zeitpunkt des Eindringens des Pollenschlauches in den ES entscheidet also, ob ein haploider oder ein diploider Embryo gebildet wird. 202 hat grosse Ähnlichkeit mit den Apomikten, nur teilt sich die Eizelle der letztgenannten durchschnittlich noch früher, auch die bei den Apomikten beobachteten Abwei-

chungen im Bau des ES finden sich in 202. Der Unterschied ist, dass in 202 der Kern der EMZ sich immer durch Meiosis geteilt hat, so dass die ohne Befruchtung gebildeten Embryonen haploid sind. Ob andere F_1-Pflanzen sich wie 202 verhalten können, oder ob einzelne der Sexuellen (die Pflanze 111) autonome Entwicklung der Eizelle zeigen können, ist nicht untersucht worden.

Wenn der Pollenschlauch in den ES der Apomikten von *P. alpina* eindringt, ist also schon ein junger Embryo gebildet worden. Dies könnte entweder auf einer frühen Teilung der Eizelle oder auf spätem Öffnen der Blüte bzw. einem langsamen Zuwachs des Pollenschlauches beruhen. Bei verschiedenen Apomikten ist die frühe Teilung der Eizelle, noch ehe die Blüte sich geöffnet hat, betont, so z. B. bei *Archhieracium* (BERGMAN, 1941). Das Vorkommen von Embryonen in ungeöffneten Blüten von den hier untersuchten Apomikten wurde beobachtet. Die Anthese und das Öffnen der Blüte ist aber offenbar verspätet, und dies bedingt, dass auch der Pollenschlauch verspätet ist. Jedenfalls war der ES entschieden älter als bei den Sexuellen, wenn man Spermakerne in ihm beobachtete. Es scheinen also beide erwähnten Momente dazu beizutragen, dass beinahe nur apomiktische Embryonen gebildet werden.

Einige neuere Abhandlungen zeigen, dass 202 nicht vereinzelt steht. KIHARA (1940) hatte eine Linie *Triticum monococcum* var. *vulgare*, die normal 0,5 % Haploide in ihrer Nachkommenschaft hatte. Frühe Kastrierung (3—4 Tage vor dem Öffnen der Blüte) und späte Pollination erhöht die Anzahl von Haploiden stark, bis 37,5 % bei der Pollination 9 Tage nach der Kastrierung. KIHARA und YAMASHITA (1938) fanden bei *T. monococcum*, andere Autoren bei anderen Objekten, dass Pollination mit röntgenbehandeltem Pollen Haploide erzeugt; dies wird von ihnen dadurch erklärt, dass die Pollenschläuche eines solchen Pollens langsam wachsen, und die alternde Eizelle sich geteilt hat, wenn der Pollenschlauch in den ES eindringt. Das Vorkommen von Endosperm in Samen mit haploiden Embryonen deutet nach KIHARA darauf, dass der Zentralkern befruchtet ist.

Einige eigentümliche Kreuzungsergebnisse in *Rubus* beruhen vielleicht auf verschiedener Zuwachsgeschwindigkeit von den Pollenschläuchen verschiedener Arten in demselben Griffel. Nach STEBBINS (l. c. S. 513) fand PETROV, dass unreduzierte Eizellen einer triploiden Form von *R. idaeus* nach Kreuzung mit *R. loganobaccus* (pentaploid) hexaploide Bastarde gibt, nach Kreuzung mit der diploiden Form von *idaeus* aber nur triploide, pseudogam entstandene Pflanzen. Dies kann

dadurch erklärt werden, dass die Pollenschläuche im letzten Fall langsam wachsen, sodass die Eizellen sich schon geteilt haben, wenn der Pollenschlauch den ES erreicht. Man gewinnt weiter den Eindruck, dass viele *Rubus*-Formen in der Samenanlage einen sexuellen, haploiden und einen aposporischen ES haben. Aposporie ist in *Rubus*-Formen festgestellt worden (THOMAS, 1940; GUSTAFSSON, 1942). In Kreuzungen, die sexuelle Bastarde geben, wuchs der Pollenschlauch relativ schnell und befruchtete die haploide Eizelle. In Kreuzungen, die pseudogame, matrokline Nachkommenschaft geben, wuchs der Pollenschlauch langsam, und die Embryobildung hatte in dem aposporischen ES begonnen, wenn der Pollenschlauch zu der Samenanlage gelangte und vielleicht einen Zentralkern befruchtete. Einige der bekannten Kreuzungsergebnisse von LIDFORSS werden vielleicht so ihre Erklärung finden.

Das Vorkommen von Zwillingembryonen wurde beobachtet. Bei den beiden Apomikten werden sie im selben ES gebildet, der eine aus einer Synergidenzelle. Dies war anscheinend einmal auch bei 202 der Fall. In den Pflanzen, die oft mehr als einen ES entwickeln, wird offenbar in der Regel nur der eine befruchtet. Es schien, dass in gewissen Fällen der unbefruchtete ES die Entwicklung des befruchteten störte, der Zeichen von Degeneration zeigen konnte. Einmal wurde doch die Weiterentwicklung von zwei ES-en beobachtet; in 202 wurden weiter zwei grosse teilungsbereite Eizellen, die eine befruchtet, die andere unbefruchtet, gefunden. 202 kann vielleicht diplo-haploide Zwillingpaare bilden, und dies sowohl aus einem einzigen wie aus zwei ES-en.

Recht auffallend ist die relative Simultanität der Zellteilungen in jungen Embryonen, die sich früh gebildet haben. Möglicherweise ist dies ein Nachklang der Simultanität der Kernteilungen im ES. Auch die Kerne der jungen Antipoden teilen sich wahrscheinlich etwa gleichzeitig. Im Protoplast des ES bleibt die Simultanität länger bestehen, die Kerne des Endosperms teilen sich recht lange gleichzeitig und wie im ES beginnen die Teilungen im chalazalen Teil.

Die regelmässige Befruchtung der Polkerne bei den Pseudogamen ist von recht hohem Interesse. Schon der erste Entdecker der sog. doppelten Befruchtung, NAWASCHIN, betonte aber, dass die Befruchtung des Zentralkerns ein wahrer Sexualakt sei, der zu der Bildung eines Nährembryos führt. Andere Forscher haben sie als eine mehr sekundäre Erscheinung betrachtet, was aber offenbar unberechtigt ist. Eine Beobachtung von dem zweiten Entdecker der Doppelbefruchtung, GUIGNARD, an *Vincetoxicum officinale* zeigt auch die Notwendig-

keit einer Befruchtung des Zentralkerns, um Endosperm bilden zu können (nach SCHNARF, 1929, zitiert). Auch in anderer Hinsicht haben neuere Untersuchungen die hohe Bedeutung der Endospermbildung gezeigt. BRINK und COOPER (1940) haben bei *Medicago sativa* die Entwicklung der Samen unmittelbar nach der Befruchtung nach Selbstung und nach Fremdbestäubung untersucht. Im ersteren Fall ist hier die Samenfertilität stark herabgesetzt. Die Entwicklung der Embryonen ist anfangs etwa die gleiche, das Endosperm entwickelt sich aber nach Selbstung schwächer und langsamer als nach Fremdbestäubung. Dies bringt es mit sich, dass das Endosperm dem sich jetzt stark entwickelnden somatischen Gewebe unterliegt und Abortion des Samens eintritt (»somatoplastic sterility»). Ein von Anfang an kräftig wachsendes Endosperm ist hier notwendig und wird durch einen nach Kreuzung erzielten Heterosiseffekt erhalten. Die erste Zeit in der Entwicklung der Samenanlage nach der Befruchtung ist also kritisch und durch Konkurrenz zwischen Endosperm und somatischem Gewebe gekennzeichnet. Wie die Fremdbefruchtung verschiedene Vorteile der aus dem Ei entwickelten Pflanze in ihrem Kampf ums Dasein zuführt, so ist sie auch für den Nährembryo in ihrem kleinen Lebensraum von grösster Bedeutung. Die Entstehung der Polkernbefruchtung und das zähe Festhalten an dieser ist also verständlich, wenn auch in vielen Fällen später Autogamie entstanden ist.

Bei autonomer Apomixis teilen sich die Polkerne, wie GENTCHEFF und GUSTAFSSON hervorheben, ohne Befruchtung. Der Pollen ist untauglich.

Bei autonomer Diplosporie sind verschiedene Erscheinungen vereint, die keine gemeinsame Ursache haben. *Poa alpina* zeigt einige von diesen. Die Eizelle hat hier bei den Apomikten sowie bei der aus der Kreuzung Sexuell × Apomikt entstandenen 202 eine Fähigkeit, sich ohne Befruchtung zu entwickeln. Sie hat dabei nicht ihre Sexualität verloren. Die Teilungsfähigkeit der Eizelle tritt bei den Apomikten durchschnittlich früher ein, als bei 202. 202 bildet haploide Makrosporen wie die sexuellen Biotypen, die Apomikten haben aber Diplosporie erworben und haben diploide Embryonen; in ihnen sind also zwei Erscheinungen vereint, die von einander unabhängig sind. Weiter hat es *P. alpina* nicht gebracht. Ein drittes Moment wäre nun, wenn die Polkerne sich ohne Befruchtung teilen könnten und ein taugliches Endosperm bildeten, d. h. eine parthenogenetische Bildung von dem Nährembryo. Dann wäre Befruchtung unnötig, und der Pollen hatte keine Aufgabe zu füllen. Mutationen, die Pollensterilität hervorrufen,

werden nun nicht durch Selektion ausgemerzt. Der schlechte Pollen vieler Apomikten kann also eine sekundäre Erscheinung sein, braucht nicht gleichzeitig mit der Apomixis erworben sein und spricht nicht unbedingt für eine Bastardierung.

Wie aus der Einleitung hervorgehen dürfte, zeigen die anderen apomiktischen *Poa*-Arten Ähnlichkeiten mit *alpina*. Bei der aposporischen *pratensis* kann eine seltene haploide Eizelle sich mit oder ohne Befruchtung entwickeln, die diploide Eizelle in dem aposporischen ES hat ihre Sexualität nicht verloren, der Zentralkern muss sehr wahrscheinlich befruchtet werden. Bei der diplosporischen *palustris* kann sich ein haploider ES bisweilen entwickeln, und anscheinend kann ihre Eizelle sich mit oder ohne Befruchtung teilen. Das Endosperm soll sich hier ohne Befruchtung bilden (KIELLANDER, 1935); nicht unwahrscheinlich ist es aber, dass eine eingehendere Untersuchung zum Auffinden von Spermakernen im ES führen wird. Pollenschläuche wurden doch beobachtet. Bemerkenswert ist, dass in *Poa* haploide, diplosporische und aposporische ES-e also sehr ähnliche Eigenschaften zeigen.

Eine dritte Form von Agamospermie ist Nuzellarembryonie. In vielen Fällen ist angegeben, dass der Reiz des Pollenschlauches für die Entwicklung der Samen notwendig oder fördernd ist (man vergleiche die Ausführungen in GENTCHEFF und GUSTAFSSON und STEBBINS). Über die Natur der Stimulation sind verschiedene Meinungen ausgesprochen worden. Es ist aber nicht unwahrscheinlich, dass eine Bildung von Endosperm notwendig ist, um die hervorsprossenden Embryonen zur Reife zu bringen, und dass in einigen Fällen die Befruchtung der Polkerne eine Bedingung der Endospermbildung ist. Bei den Orchideen kann aber die Kombination Nuzellarembryonie—Pseudogamie nicht in dieser Weise erklärt werden, da sie kein Endosperm haben. Hier fördert vielleicht die Pollination durch eine Einwirkung auf die Frucht- (und Samen-) Entwicklung indirekt die Embryobildung (GENTCHEFF und GUSTAFSSON, 1940 b).

SUMMARY.

The embryology of sexual and apomictic strains of *Poa alpina* is studied; the material is from the cultures of Professor A. MÜNTZING (*vide* MÜNTZING, 1940).

In the sexual strains the macrospore mother cell divides, forming haploid macrospores. The four macrospores may be arranged in many different ways. Often more than one macrospore germinates, and more

than one mature embryo sac is rather often observed in the nucellus. Fertilization is in the usual way. Some anomalies are described.

Two apomictic strains, K. and G., have been investigated. The nucleus of the macrospore mother cell divides late, the division is mitotic, and no macrospores are formed (diplospory as in *Antennaria*). The embryo sac is eight-nucleate, the egg cell may divide rather early. Some irregularities may occur as three polar nuclei, a synergid embryo, antipodes at the micropylar end. The embryo develops quite normally, when the pollen tube enters it has often 8—12 cells.

One of the synergides is rather disorganized at the fertilization. The male nuclei are then observed between the embryo and the cytoplasm of the embryo sac. One male nucleus penetrates into the cytoplasm and fuses with the polar nuclei, the other degenerates. The endosperm is pentaploid, the embryo diploid. The apomicts are pseudogamous, when no fertilization occurs the polar nuclei do not divide and the embryo does not reach maturity. The egg cell may presumably be fertilized but as the egg cell divides early and the flower opens late this seldom occurs.

Some F_1 plants from the cross sexual $\times$ apomict were investigated. The formation and behaviour of the macrospores are as in the sexuals. The clone 202 throws many (15 %) haploid plants. It is shown that the egg cell often divides early, and that if no pollination occurs presumably all egg cells divide. The moment at which the pollen tube enters the embryo sac decides if a haploid or a diploid embryo is formed, the polar nuclei, however, must be fertilized. The behaviour of this plant is rather like that of the apomicts.

It is suggested that the rate of growth of the pollen tube is an important factor in plants where the egg cell may divide, whether it is fertilized or not, and also where the nucellus has a haploid and an aposporous embryo sac. The importance of the fertilization of the polar nuclei is emphasized.

ZITIERTE LITERATUR.

1. BĚLAŘ, K. 1924. Die Cytologie der Merospermie bei freilebenden *Rhabdites*-Arten. — Zeitschr. f. Zellen- u. Gewebelehre 1.
2. BERGMAN, B. 1941. Studies on the embryo sac mother cell and its development in *Hieracium* subg. *Archhieracium*. — Svensk Bot. Tidskr. 35.
3. BRINK, R. A. u. COOPER, D. C. 1940. Double fertilization and the development of the seed in angiosperms. — Bot. Gazette 102.

4. EDMAN, G. 1931. Apomeiosis und Apomixis bei *Athraphaxis frutescens*. — Acta Horti Bergiani 11.
5. FAGERLIND, F. 1940. Die Terminologie der Apomixis-Prozesse. — Hereditas XXVI.
6. GENTCHEFF, G. u. GUSTAFSSON, Å. 1940 a. The balance system of meiosis in *Hieracium*. — Hereditas XXVI.
7. — u. — 1940 b. Parthenogenesis and pseudogamy in *Potentilla*. — Botaniska Notiser.
8. GERASSIMOWA, HELEN. 1934. Fertilization in *Crepis capillaris*. — La Cellule XLII.
9. GUSTAFSSON, Å. 1942. The origin and properties of the European blackberry flora. — Hereditas XXVIII.
10. KIELLANDER, C. L. 1935. Apomixis bei *Poa serotina*. — Botaniska Notiser.
11. — 1937. On the embryological basis of apomixis in *Poa palustris* L. — Svensk Bot. Tidskr. 31.
12. — 1941. Studies on apospory in *Poa pratensis* L. var. *alpigena* (FR.) LINDM. — Svensk Bot. Tidskr. 35.
13. — 1942. A subhaploid *Poa pratensis* L. with 18 chromosomes and its progeny. — Svensk Bot. Tidskr. 36.
14. KIHARA, H. 1940. Formation of haploids by means of delayed pollination in *Triticum monococcum*. — Botan. Magazine LIV.
15. KIHARA, H. u. YAMASHITA, K. 1938. Künstliche Erzeugung haploider und triploider Einkornweizen durch Bestäubung mit röntgenbestrahltem Pollen. — Commemoration papers on agronomy, prep. in honour of Prof. MASAO AKEMINE.
16. MÜNTZING, A. 1933. Apomictic and sexual seed formation in *Poa*. — Hereditas XVII.
17. — 1940. Further studies on apomixis and sexuality in *Poa*. — Hereditas XXVI.
18. NOACK, K. L. 1939. Über *Hypericum*-Kreuzungen. VI. Fortpflanzungsverhältnisse und Bastarde von *Hypericum perforatum* L. — Zeitschr. f. ind. Abst.- u. Vererb.-lehre 76.
19. PACE, LULA. 1913. Apogamy in *Atamosco*. — Bot. Gazette 56.
20. ROSENBERG, O. 1930. Apogamie und Parthenogenesis bei Pflanzen. — Handb. d. Vererb.-wiss., Berlin.
21. SCHNARF, K. 1929. Embryologie der Angiospermen. — Handb. d. Pflanzenanatomie, Berlin.
22. SOUÈGES, R. 1924. Embryogénie des Graminées. Développement dé l'embryon chez le *Poa annua* L. — C. R. acad. Paris 178.
23. STEBBINS, JR., G. L. 1941. Apomixis in the angiosperms. — Botan. Review 7.
24. TINNEY, F. W. 1940. Cytology of parthenogenesis in *Poa pratensis*. — Journ. of agricult. Research 60.
25. THOMAS, P. T. 1940. Reproductive versality in *Rubus*. II. The chromosomes and development. — Journ. of Genetics 40.
26. WEBBER, J. M. 1940. Polyembryony. — Botan. Review 6.
27. ÅKERBERG, E. 1942. Cytogenetic studies in *Poa pratensis* and its hybrid with *Poa alpina*. — Hereditas XXVIII.

THE SEPARATION OF CHROMOSOMES IN TRIPLOIDS

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IN diploids, after chiasma formation at the first meiotic division, the two chromosomes of a pair are held together by chiasmata and as a consequence they orientate themselves upon the spindle, so that at anaphase a normal reduction occurs, and thus also, from a genetical point of view, a normal segregation will result. (Cf. DARLINGTON, 1937.) In triploids, where three members of each chromosome are present, two chromosomes separate from one chromosome at anaphase, and it may be asked whether the same mechanism governs the process. This would mean that in cases where chiasmata are formed between only two chromosomes, these two chromosomes must go to opposite poles. When, on the other hand, all three chromosomes take part in chiasma formation, the question arises how the chromosomes, in their movements, answer to these circumstances. These questions may be attacked by genetical means with the aid of *Drosophila* triploids with marked chromosomes. A classical experiment on such triploids has been made by BRIDGES and ANDERSON (1925), and the following note is a study of their figures. As far as is known to the present writer, these figures have not before been analysed from this point of view.

In their experiments BRIDGES and ANDERSON made use of triploid females of the following X-chromosome constitution: *y t f/rb m B / sc bi lz dy*. For each of the several genes in one of the chromosomes there is found one gene in another chromosome lying very close by. Thus the total length of the chromosomes has been divided into 5 intervals marked by the points *y*, *sc* (0,0, 0,0); *bi*, *rb* (6,9, 7,5); *t*, *lz* (27,5, 27,7); *m*, *dy* (36,1 36,2); *f*, *B* (56,8, 57,0); and the right (proximal) end of the chromosomes. In addition, the triploids were homozygous with respect to *cv* in order to make it possible to pick out all exceptional daughters, i. e. diploid daughters, having inherited both their X's from their mother and a Y from their father. In all, 182 such daughters were found and outcrossed in order to determine their genetical constitution. Using the same symbols as employed by BRIDGES and ANDERSON, *a*

may represent a gene from any given chromosome, and *b* and *c* represent a gene from either of the other two chromosomes; *a*, *b*, and *c* may also represent the three different strands. By tabulating the exceptional daughters according to the interval in which the rightmost (the most proximally situated) recovered crossing over occurred, we get Table 1. (This is, with some small rearrangements, Table 13 from BRIDGES and ANDERSON, which has been compiled from their Table 6.)

It is obvious that after chiasma formation there will occur crossover as well as non-crossover chromosomes. Among the 84 non-crossovers there may thus be several (perhaps all) which have arisen after chiasma formation, but they escape identification from this point of view.

The exceptional daughters of type *aa*/*bc* have arisen after

TABLE 1. *Distribution of the exceptional females. (Cf. text.)*

Type of female	Interval					
	1	2	3	4	5	Total
Non-crossing over *a*/*b*						84
Crossing over *aa*/*bc*	19	24	7	23		73
» » *aa*/*ab*	10	4	2	1		17
» » *ab*/*ba*		1	1	3		5
» » *ab*/*bc*			1			1
» » *a*/*a*					2	2

separation of the two chromosomes, which have formed a chiasma. They are 73 in number and fairly equally distributed in the four leftmost intervals. Of these 73 females, 25 showed still one or more crossings over to the left, — in 12 flies the non-rightmost crossing over was also between *b* and *c*, whereas in 13 flies this was not the case.

Females of the type *aa*/*ab*, 17 in number, occur after non-separation of the two chromosomes which have formed a chiasma. But their numbers decrease from the leftmost interval to the rightmost. Table 1 is made up according to the rightmost point of crossing over, but of course only with regard to the recovered crossovers. It is thus quite possible that the 10 *aa*/*ab* females with crossing over in interval 1 occurred after still another chiasma farther to the right, for instance between *b* and *c*, but of such a kind that no crossing over chromosome was recovered. In this way it is *possible* that also concerning these 10 females the rightmost chiasma governed the chromosome separation. Of the remaining 7 females *aa*/*ab* the same may be true, but the probability is smaller, 4 of them did not show any further crossing

over to the left, 1 had a crossing over between *a* and *b*, and 2 had a crossing over between *a* and *c*. The formula written in full of the last of these is, for instance, *aaaaa/ccaab*. It does not seem very probable that a chiasma (without recovered crossing over) situated still more to the right should have governed the mode of separation. It seems more likely that the orientation upon the spindle in this case is due to the leftmost chiasma.

The 5 *ab/ba* females must also have occurred after non-separation of the chromosomes taking part in chiasma formation. Four of them did not show any further crossing over, whereas one, belonging to interval 3, had a further crossing over in interval 1, but this was also between *a* and *b*. These 5 cases may perhaps also be explained by another chiasma without recovered crossing over.

The single female *ab/bc* did not show any further crossing over. In this case two chiasmata must have been formed within the same interval, one between *a* and *b*, and one between *b* and *c*. One of them has governed the orientation, but it is impossible to determine which is the proximal one.

Finally, the two *a/a* females have occurred after a non-separation of the two chromosomes taking part in chiasma formation in interval 5. It is very unlikely that a non-recovered chiasma situated more to the right should have governed the separation, but a more distally situated chiasma may have done it.

Summing up, in 73 out of 98 cases, i. e. in the great majority of the cases, it is directly observable that those two chromosomes separate between which the most proximally situated chiasma is formed. In a number of other cases it may be assumed that chiasmata situated proximally to the observed point of crossing over, but without recovered crossover chromosomes, have governed the mode of separation. In still a few other cases it seems probable that a chiasma, but not the most proximal one, has governed the direction of separation. It is, however, impossible to prove, or to make it very probable, that a chiasma formation always determines the direction of anaphase separation. It seems highly desirable to get a larger material in order to make a more thorough investigation of this interesting question.

LITERATURE CITED.

1. BRIDGES, C. B. and ANDERSON, E. G. 1925. Crossing over in the X-chromosome of triploid females of *Drosophila melanogaster*. — Genetics 10: 418—441.
2. DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd ed. — London.

ANEUPLOIDY AND SEED SHRIVELLING IN TETRAPLOID RYE

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FIVE years ago an American variety of tetraploid rye, produced by DORSEY (1936) by means of the heat shock method, was kindly sent to me. Together with other tetraploid rye varieties later on produced with colchicine this American tetraploid variety was studied with regard to morphology, fertility and chromosomal variation. In this report the apparent production of parthenogenetic diploids and the correlation between aneuploidy and seed shrivelling will be briefly considered.

I. APPARENT REVERSIONS TO DIPLOIDY.

Like other autotetraploids the tetraploid rye is not cytologically stable, a certain proportion of aneuploids being formed besides euploid plants with $2n = 28$. In the summer of 1938 13 tetraploid plants were available, which had been controlled to have $2n = 28$ or approximately this number. The seeds harvested from these plants were mixed and from this mixture 600 seeds were sown directly in the field. A hundred seeds were sown in pots, root tips being fixed from the 62 plants resulting. Of these plants 53 were found to have $2n = 28$, 5 had $2n = 27$, and 4 plants $2n = 29$. This gives a percentage of aberrants of $14{,}52 \pm 4{,}47$. The plants cytologically examined did not winter well and, thus, in the summer of 1939 the seeds for next generation were taken from the field material. Among these plants one individual had been observed to differ conspicuously from the rest and was later on found to be a triploid. Evidently this plant was the result of a spontaneous cross between one of the tetraploids grown in 1937 and an adjacent diploid rye plant. The other field plants were harvested separately, and during this work it was observed that two types of kernels could be distinguished, viz. the big ordinary kernels and smaller, very thin ones with a poor germination capacity. Though these two categories were not sharply separated they could be distinguished in 63 plants among a total of 274 plants available. In the autumn of

1939 the good seeds of all the plants were mixed and from the mixture a sample was taken in order to determine the average variation in chromosome number of the material. In the same way an average sample of the poor seed category was germinated. The result was 85 plants from good kernels and 76 plants from poor ones. The chromosome numbers of these plants were determined with the following result (Table 1).

TABLE 1. *Kernel quality and chromosome number in average samples of tetraploid rye.*

Year	Kernel quality	Chromosome number															n	Total percentage of aberrants	Percentage of tetraploid aberrants
		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
1939—1940 ...	Good	1	1	1	—	—	—	1	—	—	—	—	—	11	60	9	1		85	29,41	25,93
»	Bad	11	1	—	—	—	4	1	—	—	—	—	1	5	41	10	2		76	46,05	30,51
1940—1941 ...	Good	6	—	—	—	—	—	—	—	—	—	—	—	2	66	16	—		90	26,67	21,43
»	Bad	9	9	2	—	1	1	2	3	—	—	—	—	21	53	11	1		113	53,10	38,37
Total, good		7	1	1	—	—	—	—	1	—	—	—	—	13	126	25	1		175	28,00	23,64
» , bad		20	10	2	—	1	1	6	4	—	—	—	—	1	26	94	21	3	189	50,26	35,17

As is evident from the table, most of the plants showed the expected variation around 28, the chromosome numbers ranging from 26 to 30. In addition to these individuals, however, there was a certain proportion of diploid, or approximately diploid, plants with 14—16 chromosomes and also a few triploid individuals having $2n = 21$ or one chromosome less. The occurrence of a low proportion of triploid plants might, indeed, be expected, as the mother plants were openpollinated and not effectively separated from plots of diploid rye growing in the vicinity. The production of plants with 14—16 chromosomes was more difficult to explain. The most plausible explanation seemed to be that they had arisen by parthenogenetic development of reduced ovules. It is true that autotetraploids of higher plants, in contrast to autopolyploid mosses, are generally stable as regards their average chromosome number

(cf. MÜNTZING, 1936, p. 320), but recently a few cases have been described which represent exceptions to this rule. Thus, tetraploid maize has been reported to produce diploids by parthenogenesis (EINSET, 1939; RANDOLPH, 1941). HERIBERT NILSSON (1939) observed the production of a diploid *Oenothera Lamarckiana* from a tetraploid strain, and according to SCHWANITZ (1941) tetraploid plants of *Lepidium sativum* have a marked tendency to revert to the diploid chromosome number. However, especially since such a behaviour was contrary to my previous experience in various autotetraploids, the apparent parthenogenetic development of diploids from tetraploid rye was studied more closely.

In the first place the experiment was repeated again in 1940, plants being raised from both kernel categories. As is evident from Table 1, the result was just the same as in the previous year, a minority of plants being exactly or approximately diploid or triploid. The appearance of some aneuploid plants, differing in chromosome number from the exact diploids and triploids, might be ascribed to the formation of aneuploid ovules by the tetraploid mother individuals. It seemed strange, however, that so many of the plants in the approximately diploid category had numbers slightly deviating from 14. Therefore, in order to settle the question of parthenogenesis in tetraploid rye, new progenies of tetraploid plants were studied again in 1941. This time no average sample from the whole plot was taken, but the kernels of single openpollinated mother plants were studied separately. The chromosome numbers of the mother plants were not known, it is true, but care was taken to select typical, vigorous plants, the less vigorous aberrants with slightly deviating chromosome numbers being avoided in this way. Thus, nineteen different progenies were raised from separate mother plants, a total of 553 plants being cytologically examined. In 16 of the progenies the plants were all tetraploid, the chromosome number ranging from 27 to 30 (Table 2). In one of these progenies there was also a single plant with $2n = 19$, all the other plants being tetraploid. This hypotriploid must be ascribed to the fertilization of an ovule with 12 chromosomes by pollen of diploid rye growing in the vicinity. The remaining two progenies (not included in Table 2) were evidently derived from diploid mother plants. One of these progenies consisted of 26 plants, which were all diploid. The other progeny, finally, comprised 21 diploids, 1 triploid and 1 tetraploid. The triploid plant must be the result of a diploid $\times$ tetraploid pollination, but the origin of the single tetraploid is more obscure. An error at the labelling of the seedlings is not entirely excluded. The

plant may also have arisen from the fertilization of an unreduced ovule of the diploid mother by pollen from the tetraploid plants surrounding the single diploid individual. At any rate, these data clearly demonstrate that the diploid plants obtained had not arisen by parthenogenesis.

Thus, if parthenogenesis ever occurs in tetraploid rye it must at least be a very rare phenomenon, which cannot account for the rather high proportion of diploids and aneuploids observed in the years 1939—1940 (Table 1). This being the case, another explanation of the production of such plants is needed. The only possibility is the segregation of diploids from triploids and triploid derivatives, arisen from occasional spontaneous hybridization with diploid rye. As mentioned above there was, indeed, one identified triploid in the plot of tetraploid rye flowering in 1939. There is reason to believe that several other triploids were also present. This view is supported by the fact that of the 274 plants available 63 plants were found to produce kernels of variable size, including the conspicuous small and thin kernels mentioned above. It is possible that all or most of these plants were triploids. In next generation such openpollinated plants with access to pollen of tetraploids as well as diploids would give rise to diploid and aneuploid progeny besides tetraploids. About the same story will also account for the occurrence of diploids and aneuploids in the material of the following year.

This explanation is also in harmony with the relatively high proportion of trisomic individuals and other aneuploids with low numbers occurring besides the true diploids with $2n = 14$. As may be seen in Table 1, a total of 14 plants with $2n = 15$ or 16 were found in the two years, at the same time as 27 plants having exactly 14 chromosomes. If these plants had arisen by parthenogenesis this would mean the occurrence of a very high frequency of aneuploid ovules in tetraploid rye. This frequency should amount to about 50 per cent, since ovules with less than 14 chromosomes would not give viable parthenogenetic offspring. However, the actual frequency of aberrants, having a number slightly deviating from 28, is much lower than 50 per cent. This is evident from the values in Tables 1—2 and from the first chromosome counts in this tetraploid material, giving a percentage of aberrants of only $14{,}52 \pm 4{,}47$ (p. 65). Thus, it is quite clear that the spontaneous diploids and aneuploids with low numbers occurring in tetraploid rye arise by hybridization and not by parthenogenesis.

II. CORRELATION BETWEEN CHROMOSOME NUMBER AND KERNEL QUALITY.

As mentioned above two types of kernels were distinguished in the average seed samples of tetraploid rye used for germination in 1939 and 1940. These two categories, »good» and »bad», were germinated separately. The resulting plants were found to show average differences in chromosome number, the plants from bad kernels more frequently having aberrant chromosome numbers than the plants from good kernels. This is evident from the data given in Table 1. According to these values the percentage of aberrants from good and bad seeds was 29,41 and 46,05 respectively in 1939 and 26,67 and 53,10 in 1940. If the values of both years are combined, the total percentage of aberrants from good seeds will be 28,00 and the percentage from bad seeds 50,26. The significance of this difference was tested with the χ^2 method. If the variates are combined in the two groups 14—21 and 26—30 the distribution will be the following:

Chromosome number:	14—21	26—30
Good seeds:	10	165
Bad » :	44	145

This distribution gives a χ^2 value of 22,19 and a P value smaller than 0,001. Thus, it is quite certain that the percentage of aberrants in this material may be increased by selection of bad seeds and *vice versa*.

This result may be explained in the following way: It is a well known fact that in crosses between parents representing different degrees of polyploidy the hybrid seeds are often shrivelled and of a more or less poor quality. In some species the hybrid seeds abort completely and are incapable of germination. Various workers have tried to explain these disturbances in different ways. For my part I have called attention to the disturbed quantitative relations between the chromosome numbers of embryo, endosperm and surrounding maternal tissues (MÜNTZING, 1930, 1933, 1936, 1941). In normal cases this relation is 2 : 3 : 2, but if the parents crossed, or the gametes uniting, have different chromosome numbers, these relations will be changed, this change having more or less severe effects.

In the present material the diploids and aneuploids with low chromosome numbers were shown to be descendants of triploids and triploid derivatives. In such mother plants the relations between the chromosome numbers of embryo, endosperm and maternal tissues will

be disturbed, and according to the theory this should result in seeds which are more or less shrivelled. It has been observed, indeed, that in triploid rye plants, experimentally produced, the seed quality is rather poor (unpublished data). Under such circumstances it is natural that a higher proportion of aberrants and diploids should be obtained from bad than from good seeds. In 1940, it is true, as many as 6 diploids were obtained from good seeds. It is rather probable, however, that the mother individuals of these plants were already diploid, and in such a case the seed development has been normal.

The difference between the »bad» and »good» categories is not only limited to a difference in the frequency of aberrant plants with low numbers but is also possible to trace in the chromosomal variation around the tetraploid number 28. The bad seeds seem to give a higher proportion of plants with the aberrant chromosome numbers 26, 27, 29 and 30 than the other category. According to Table 1 there was a total of 126 plants with $2n = 28$ in the »good» category and 39 plants with the aneuploid numbers 27, 29 and 30. In the »bad» category 94 plants had $2n = 28$ and 51 plants the deviating numbers 26, 27, 29 and 30. A test of the distributions 126 : 39 and 94 : 51 by means of the χ^2 method gave a χ^2 value of 4,98, the corresponding P being situated between 0,05 and 0,02. Thus, though not quite conclusive, the data indicate rather strongly that the bad seeds give a higher proportion of tetraploid aberrants than the good seeds.

This question was further studied in the material raised in 1941. The 17 progenies from tetraploid mother plants were divided into two groups. In the former group, consisting of 6 progenies, all the seeds of each mother plant were used for germination. In this group it was found to be convenient to divide the kernels into three categories, good, bad and medium. In the other group of material consisting of 11 progenies not all the seeds of each mother plant were germinated but only those representing the extremes. Thus, from each plant about 20 quite good seeds were selected and, on the other hand, as many really bad seeds as were available. The number of such seeds, however, was rather low and ranged from 4 to 15. Germination in this category was poor, of course, but nevertheless a sufficient number of plants could be raised for comparison with plants belonging to the other category. The chromosome numbers of all the plants were determined, the total result being given in Table 2.

In the selection group, in which only the very best and the quite poor seeds had been germinated, these two categories were found to

TABLE 2. *Kernel quality and chromosome number in progenies of separate mother plants of tetraploid rye.*

	Kernel quality	Chromosome number 19 ... 27	28	29	30	n	Percentage of aneuploids
Kernels especially selected	Good	15	166	16	1	198	16,16
	Bad	1 — 14	23	1	1	40	42,50
All kernels germinated	Good	12	148	19	5	184	19,57
	Medium	6	37	8		51	27,45
	Bad	7	22	1	1	31	29,03
	Total	25	207	28	6	266	22,18

differ significantly as regards the chromosomal variation of the resulting plants. The poor seeds evidently give a higher proportion of aneuploids than the good seeds. If all aneuploids are added the result will be as follows:

	2n = 28	Aneuploids	Percentage of aneuploids
Selected good seeds:	166	32	16,16
» bad » :	23	17	42,50

A calculation of χ^2 gives the value 14,12, which corresponds to a P smaller than 0,001. Thus, the two distributions are certainly different. Evidently this result implies that *in tetraploid rye it is possible by seed selection to diminish the frequency of aneuploid aberrants among the plants raised.* The same thing is probably true of other cytologically unstable polyploid cereals as well, such as tetraploid barley and wheat-rye amphidiploids. As the aneuploid aberrants are less fertile and vigorous than the euploid plants, such seed selection may in certain cases be of practical importance as a means to increase the yield of the material sown.

The tendency of bad seeds to give aneuploid plants may again be explained as due to disturbed quantitative relations between the chromosome numbers of embryo, endosperm and maternal tissues. In the cases discussed above, in which apparently parthenogenetic individuals with low chromosome numbers were segregated from triploids or triploid derivatives, the quantitative disturbances were rather pronounced. It is more remarkable that the slight chromosomal variation occurring

around the tetraploid number is sufficient to influence the quality of the seeds in a noticeable way.

So far only those progenies have been considered which were raised from especially good or bad seeds. In the other group of material, in which all the seeds of each mother plant were germinated, the contrast between the good and the bad seed category was less extreme. However, also in this case the chromosome numbers seem to be influenced in the same way as before. According to Table 2 the percentage of aneuploid aberrants in the whole of this group of material is 22,18. However, among the plants raised from good seeds the corresponding value is 19,57, and among the plants from bad seeds it is 29,03. The »medium» seeds gave 27,45 per cent of aberrants. If all aneuploids are added to one category the following distributions will be obtained:

	2n = 28	Aneuploids	Percentage of aneuploids
Good seeds:	148	36	19,57
Medium » :	37	14	27,45
Bad » :	22	9	29,03

A calculation of χ^2 for these distributions does not reveal any significant difference, χ^2 being 2,40 and P = 0,30. However, the results point exactly in the same direction as in the other group of material, and therefore the present values may be considered to support the previous significant data.

So far we have considered the correlation between seed quality and total percentage of aneuploids. However, it seems possible to make a further differentiation. During the statistical treatment of the values it was observed that aberrants with chromosome numbers lower than 28 are relatively more frequent among the plants raised from bad seeds than among those raised from good seeds and *vice versa*. Considering first the material raised from especially selected seeds (Table 2, upper part), the good seeds produced 17 aberrants with numbers higher than 28 (2n = 29 and 30) and 15 aberrants with lower numbers (2n = 27). The bad seeds on the contrary gave only 2 aberrants with numbers higher than 28 but 15 aberrants with lower numbers. A comparison of the distributions 15 : 17 and 15 : 2 by means of the χ^2 method gave a χ^2 value of 8,00. This corresponds to a P value situated between 0,01 and 0,001. Thus, it is almost quite certain that *seeds containing hypotetraploid embryos are on an average less well developed than seeds containing hypertetraploid embryos.*

This conclusion was also supported by the other group of material (Table 2, lower part), in which all the seeds of each mother plant had been germinated. However, as selection in this group had been less rigid than in the previous group, the average differences between the frequencies of hypo- and hypertetraploid were also less pronounced. Before testing the distributions with the χ^2 method the low absolute numbers of aberrants makes it necessary to add the values in the categories »bad» and »medium». In this way the category »bad + medium seeds» will contain 10 hypertetraploid and 13 hypotetraploid aberrants, the corresponding values of the category »good» being 24 hypertetraploid and 12 hypotetraploid plants. A test of the distributions 12 : 24 and 13 : 10 gave a χ^2 of 3,09, which corresponds to a P situated between 0,10 and 0,05. Thus, in this case the difference is not significant. However, since the difference goes in the same direction as before it may be correct to add these and the previous values to the following total distributions, representing all the aberrants given in Table 2.

	Hypotetraploid	Hypertetraploid
Good seeds:	27	41
Bad + medium seeds:	28	12

In this case χ^2 will be 9,248, which again corresponds to a P value situated between 0,01 and 0,001. The χ^2 values exactly corresponding to $P = 0{,}01$ and 0,001 are 6,635 and 10,827 respectively. Thus, the present P value is closer to 0,001 than the P value corresponding to $\chi^2 = 8{,}00$. As just stated, this value was obtained for the hypo- and hypertetraploids obtained after a rigid seed selection. Thus, the total data available leave no reasonable doubt as to the significance of the phenomenon observed.

At our present state of knowledge it is not possible to give any convincing explanation of the fact that hypo- and hypertetraploid embryos do not occur with the same relative frequency in good and bad seeds. It may be a weak expression of the same causes as those underlying the fact that monosomic diploids are not viable, in contrast to trisomic individuals. In autotetraploids there is less reason for such differences between hypo- and hyperploid individuals. However, one case observed by the present writer in *Galeopsis* is of interest in this connection. As already reported (MÜNTZING, 1941, p. 198), hypertetraploid aberrants in an autotetraploid strain of *G. pubescens* are significantly more frequent than hypotetraploid plants. The difference in this

case was very marked, the plants in the former category being more than four times as frequent as those in the other category. This excess was supposed to be due to a strong selection especially among the gametes. In view of the present results in rye it seems more likely that the hypotetraploid category is to a large extent eliminated during the seed development. In rye the hypotetraploid category is more viable, but its poor constitution is expressed in the poor quality of the seeds.

Though it is not yet possible to explain why hypertetraploid embryos are present in better seeds than hypotetraploid ones, the general idea of a delicate balance between the different tissues in contact during seed development is strongly supported by recent results of BRINK and COOPER (1939, 1941) and COOPER and BRINK (1939). According to these authors, continued development of the young seed apparently requires that the endosperm maintain an ascendant physiological position relative to the growing maternal tissue adjacent to the embryo sac, with which it must compete for a limited food supply. Otherwise the partition of nutrients becomes unbalanced in favour of the nucellus or integument and so-called »somatoplastic sterility» may result. Such disturbances were observed in inbred material and in species crosses, and were especially pronounced in the species cross *Nicotiana rustica* ($2n = 48$) $\times$ *glutinosa* ($2n = 24$). It is rather obvious that changes in the normal chromosome number ratio 2 : 3 : 2 of the embryo, endosperm and maternal tissues will easily lead to disturbances of the kind observed by BRINK and COOPER. As demonstrated above, such disturbances are not only limited to gross changes in the chromosome number ratios, e. g. following hybridization between diploids and tetraploids, but also occur as a result of the slight chromosomal variation occurring in an autotetraploid.

LITERATURE CITED.

1. BRINK, R. A. and COOPER, D. C. 1939. The significance of double fertilization in flowering plants. — Records of the Genetics Soc. of America, No. 8, p. 113.
2. — and — 1941. Incomplete seed failure as a result of somatoplastic sterility. — Genetics, Vol. 26, No. 5, pp. 487—505.
3. COOPER, D. C. and BRINK, R. A. 1939. Somatoplastic sterility as a cause of seed failure following interspecific hybridization. — Records of the Genetics Soc. of America, No. 8, p. 114.
4. DORSEY, E. 1936. Induced polyploidy in wheat and rye. — Journal of Heredity, Vol. 27, No. 4, pp. 155—160.

5. EINSET, J. 1940. Characteristics of parthenogenetic diploids derived from tetraploid maize. — Records of the Genetics Soc. of America, No. 9, p. 150.
6. MÜNTZING, A. 1930. Über Chromosomenvermehrung in *Galeopsis*-Kreuzungen und ihre phylogenetische Bedeutung. — Hereditas XIV, pp. 153—172.
7. — 1933. Hybrid incompatibility and the origin of polyploidy. — Hereditas XVIII, pp. 33—55.
8. — 1936. The evolutionary significance of autopolyploidy. — Hereditas XXI, pp. 263—378.
9. — 1941. New material and cross combinations in *Galeopsis* after colchicine-induced chromosome doubling. — Hereditas XXVII, pp. 193—201.
10. NILSSON, HERIBERT. 1939. Eine diploide Form aus der tetraploiden *Oenothera gigantea*. — Hereditas XXV, pp. 1—8.
11. RANDOLPH, L. F. 1941. An evaluation of induced polyploidy as a method of breeding crop plants. — Am. Naturalist, Vol. LXXV, pp. 347—363.
12. SCHWANITZ, F. 1941. Untersuchungen über den Ertrag getriebener diploider und tetraploider Gartenkresse (*Lepidium sativum*). — Der Züchter, Bd. 13, pp. 155—160.

POLYMERISM IN MORBID INHERITANCE

EXPERIMENTAL INVESTIGATIONS ON A MULTI-FACTOR HEREDITARY DISEASE IN MICE

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IN the case of hereditary diseases a great many investigators have supposed the manner of inheritance to be polymeric, multi-factor, conditioned by several genes. This hypothesis has especially been advanced in the cases in which the evidence of Mendelian inheritance is strong, but the data do not bear out this view if a single dominant or a single recessive factor is assumed. Numerous examples may be mentioned of this fact.

Thus PLATE writes in his text-book, 1913, that hereditary deaf-mutism is a trihybrid trait brought about by one recessive and two dominant factors. DAHLBERG (1931), on the basis of FAY's investigations, has developed PLATE's hypothesis further by saying that in his opinion no less than three dominant and one recessive gene must be active at the development of hereditary deaf-mutism. In the opinion of BAUER and STEIN (1926) otosclerosis is due to dimeric recessive genes.

In 1916 RÜDIN advanced the theory of dimeric recessivity in the case of schizophrenia, and later LEWIS declared as his opinion that the manic-depressive psychosis was conditioned by two recessive and one dominant gene.

In the case of oligophrenia the manner of inheritance has likewise been supposed to be polymeric (ROSANOFF), and as to mongoloid idiocy MACKLIN (1929), on the basis of the twin examinations in hand, even goes to the length of presuming that this affection is brought about by five recessive genes, or by two dominant and four recessive genes. [KEMP has pointed out (1941) that the twin examinations in connection with mongoloid idiocy are suggestive of a dominant manner of inheritance with frequent appearance by mutation.]

As to various nervous diseases many writers have supposed the manner of inheritance to be multi-factor. Thus progressive muscular dystrophy is by HANSEN and v. UBISCH (1927) supposed to be conditioned by two dominant genes, by MINKOWSKI and SIDLER (1927—28) by two recessive genes, by SJÖVALL (1936; in agreement with DAHLBERG) likewise by two recessive pairs of genes, one of which is possibly sex-linked. But the latter author points out at the same time that the possibility of a mixture of different ways of inheritance cannot be excluded.

CURTIUS (1933) supposes the multiple sclerosis to be conditioned by polymeric genes, whereas THUMS (1936) maintains that the disease is not hereditary. CURTIUS points out that cases of allergic diathesis, eczema, vegetative lability on the whole, senile dementia, psychopathy, suicide, manic-depressive psychosis, epilepsy, and enuresis occur frequently in the families of these patients. This leads CURTIUS to

the supposition that multiple sclerosis is a rare syndrome conditioned by polymeric genes, which when acting separately may bring about manifold neuropathologic symptoms, but which jointly develop multiple sclerosis. The development of this disease is, however, also dependent both on modifying genes and on exogenous causes. In this connection CURTIUS calls attention to the fact that similar observations have been made in the case of epileptic families, but that, on account of selective choice of conjugal partner, there would be some sense in also regarding these families as accumulators of the most varied pathologic dispositions.

Also in the case of a number of other diseases the manner of inheritance has been supposed to be dimeric or polymeric, e. g. LAURENCE—MOON—BIEDL's syndrome, allergia, and harelip-cleft palate. As to the last-mentioned disease, however, FOGH—ANDERSEN's (1942) investigations have shown that there is no reason for assuming polymerism.

From the above statements it appears that previously the dimeric or polymeric manner of inheritance was generally regarded to be frequent in the case of hereditary diseases.

Of recent years this view has, however, been contested from various quarters.

Thus LENZ maintained even as far back as many years ago that hereditary diseases are nearly always monomeric, whereas normal traits are most frequently to be regarded as polymeric. He is supported in this view by CSIK and MATHER (1937—38), who have made a statement on the investigations in hand on the following four diseases: oligophrenia, LAURENCE—MOON—BIEDL's syndrome, harelip and cleft-palate, and allergy, which all show an excess of affected males, and in the case of which accordingly the manner of inheritance was previously supposed to be dimeric or polymeric with sex-linkage of one of the controlling genes. CSIK and MATHER have now shown that in no case can sex-linked genes reasonably be supposed to be concerned in their inheritance, a fact which also puts an end to the hypothesis of polymerism.

ROBERTS (1940) has pointed out, in agreement with LENZ (1936), that polymeric inheritance is hardly in force in the case of hereditary diseases, whereas no doubt normal traits often show multi-factor inheritance.

In animals and plants many traits have been observed which depend upon the interaction of two, or at most a few, gene pairs. In most cases, however, the two gene pairs do produce effects when observed independently, the interaction consisting in the appearance of yet another effect, when genes of the two pairs are present in certain combinations. Such genes are, however, but seldom truly complementary, in the sense that they do not when alone produce any effect.

But as to pathologic complementary genes in man we must presume that each of the complementary genes is, when appearing alone, without any demonstrable effect, because in man more than in animals and plants any abnormal variant will practically always be observed.

There is no doubt that homologous polymeric factors play a considerable part in the normal variation. Normal traits are nearly always multi-factor. Also in man but few examples are known of normal traits inherited by simple manner of inheritance: the blood-group traits, the secreter—non-secreter phenomenon (according to blood-groups), the power of tasting phenyl-thiocarbamide, and possibly the colour of eyes and hair. And with regard to the blood types we may even regard types A and B as anormal in so far as they developed formerly by mutation of type 0.

According to a modern view we should then regard most normal traits as polymeric, and the pathologic traits as monomeric. ROBERTS (1940) has pointed out that in man it seems most expedient to distinguish between (1) the traits in which a single gene is wholly responsible, or in which the presence of a single gene is a necessary condition for the appearance of the trait, (2) those in which the genetic constitution is wholly or partly responsible, but in which the effect is due to the combined action of many genes, the action of single genes not being individually distinguishable. In conformity with this we know but very few human cases in which the effect is due to a combined action of two or three individually distinguishable genes.

The normal characters in man are very often graded characters, the inheritance of which is multi-factor; many genes are concerned, each one alone produces a small effect, the total result being due to their combined action. When the pathologic gene develops by mutation, it produces its effect alone, naturally always in relation to the gene milieu present in each single individual. Gradually the pathologic gene is counteracted by various factors: natural selection, increased occurrence in the kind in question of modifying genes, counteracting it or quite suppressing it. Soon after its appearance it will manifest itself in the less stable heterozygous condition (dominant or intermediary), later also in the more stable homozygous condition (recessive). The relation of other genes to the pathologic gene is limited to modification or suppression of its effect. It is not very likely that an abnormal gene should establish some system of interaction with another single gene of large effect or with two or three other genes. But the pathologic gene does not differ from the normal in

any fundamental way, only by its larger effect. Alone it manifests itself plainly, unlike most of the genes bringing about normal traits, which must be regarded as »weak» when alone.

Also within medical genetics widely different views have been advanced with regard to the importance of polymerism or multi-factor inheritance for the appearance of hereditary diseases. Naturally it would be of great value for genetic pathology to have this important question elucidated further. The author has therefore tried to cross-breed two strains of mice in which there had occurred monomerically hereditary diseases, in one strain with dominant, in the other with recessive, manner of inheritance.

The disease with the recessive manner of inheritance was anterior pituitary dwarfism, an affection which has been described by many writers, first by SNELL and SMITH & MAC DOWELL, and has later been investigated very thoroughly by a scientific team in Copenhagen (KEMP, KEMP and MARX, BARTELS, MØLLENBACH, GRUNNET, and FRANCIS). The disease has always shown a typical monomeric recessive manner of inheritance, so that the heterozygotes are exactly like the normal. The animals that have the hereditary disposition homozygously display a pronounced hypoplasia of their anterior pituitary gland, accompanied by dwarfism, these animals seldom weighing more than 5 g., and never more than 10 g., whereas the corresponding normal animals weigh between 20 and 30 g.

The disease with the dominant manner of inheritance was found in a strain that has been submitted to experiments for very long, and was described already by CUÉNOT in 1905. Some of the mice of this strain are yellow, and some are grey. The gene that brings about the yellow coat is dominant with a recessive lethal effect, so that those animals which get the gene homozygously will die early in their fetal life, and of the offspring from two heterozygotes $^1/_3$ will be grey and $^2/_3$ yellow. It appears now that the yellow mice are also disposed to hereditary adipositas, so that they gradually reach a weight of 70 to 80 g. By far the greater part of the increase in weight is due to fat deposited rather plentifully in all tissue and organs. A detailed description of this strain of mice was given recently in a monograph by WEITZE (1941).

An attempt was now made to join these two pathologic genes in one individual. The dominant gene for adipositas will hereafter be marked by the letter *a*, its allelomorph by *A*. The recessive gene for dwarfism will be marked by *d*, and its allelomorph by *D*.

By cross-breeding two heterozygotes for dwarfism we get:

$$Dd \times Dd = DD + 2Dd + dd,$$

which means that $^1/_4$ of the offspring are dwarfs.

By cross-breeding two yellow mice with adipositas we get:

$$Aa \times Aa = AA + 2Aa + aa,$$

aa die, and 2*Aa* become yellow and get adipositas.

Cross-breeding of a phenotypically fat mouse *Aa* with a heterozygote for dwarfism *Dd*

AaDD × *AADd* gives the following gametes:

AD, *aD* and *AD*, *Ad*, from which we get the following gamete combinations:

AADD: normal
AaDD: normal with adipositas
AADd: heterozygote for dwarfism
AaDd: heterozygote for dwarfism with adipositas.

By cross-breeding two heterozygotes for dwarfism with adipositas we get:

AaDd × *AaDd*, which may form the following gametes:
AD, *Ad*, *aD*, *ad* and
AD, *Ad*, *aD*, *ad*, which give the following gamete combinations:
1*AADD*: normal, no heterozygote
2*AADd*: normal, heterozygote for dwarfism
1*AAdd*: dwarf without adipositas
2*AaDD*: mice with adipositas without disposition to dwarfism
4*AaDd*: heterozygotes for dwarfism with adipositas
2*Aadd*: dwarfs with adipositas
1*aaDD*: not viable
2*aaDd*: not viable
1*aadd*: not viable.

It may be seen from this that $^2/_{16}$ of the offspring produced by the cross-breeding of two heterozygotes for dwarfism with adipositas get the formula of *Aadd*. In other words, these animals are homozygous with regard to their disposition to dwarfism and heterozygous with regard to their disposition to adipositas.

The dwarfs with adipositas present a new and not previously observed pathologic picture. It may be described rather as a summation of the two original pathologic pictures (see Fig. 1). The skeleton of these animals showed them to be dwarfs, the length of body and tail

did not differ from that which we find in dwarfs with no disposition to adipositas, nor did the endocrine glands or other internal organs differ essentially from those found in ordinary dwarfs (a detailed histologic examination of these organs will be published elsewhere). But these animals were easily distinguishable by their yellow coat, and gradually they also developed adipositas. The adipositas appeared at different ages and varied somewhat in degree (see Table 1); in the most pronounced cases these dwarfs reached a weight of 10 to 20 g., whereas

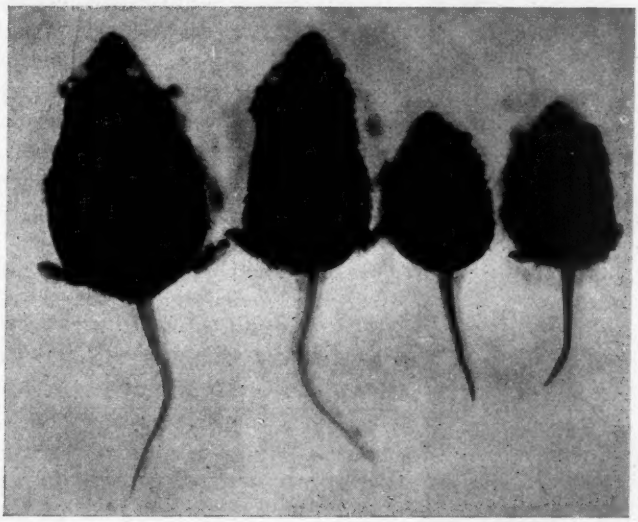

Fig. 1. To the left a female mouse (weight 68 g.), a heterozygote for dwarfism with adipositas. To the right 3 of its young ones; the male was a heterozygote for dwarfism and suffered from adipositas. No. 2 from the left has adipositas, but is no dwarf (weight 40 g.). Nos. 3 (weight 20 g.) and 4 (weight 20 g.) are homozygous for dwarfism and have adipositas.

the weight of the ordinary dwarfs was 5 to 10 g. (Table 2). Enormous amounts of fat were deposited between the organs (Fig. 2), and the whole habitus of the animals was marked by the excessive adipositas. They would mostly sit still, very unwilling to move, and when they did move it caused them trouble.

From Table 1 it may be seen that the weight of dwarfs with adipositas varies considerably, whereas Table 2 shows that in the case of dwarfs without adipositas the weight is more constant. This accords well with the experience generally made that there is a comparatively

great constancy in the manifestation of homozygous recessive genes, whereas the dominant genes are more varying in their manifestation.

TABLE 1. *The weight of 8 dwarfs (in g.) with adipositas (4 ♂ and 4 ♀) by the end of each of their first 12 months of life.*

	♂ 201	♂ 205	♂ 49	♂ 66	♂ Average	♀ 82	♀ 206	♀ 192	♀ 273	♀ Average
1:st month	4,0	7,0	—	—	5,5	4,5	6,5	4,5	5,0	5,1
2:nd »	5,5	8,0	—	—	6,8	7,5	7,5	5,0	5,0	6,3
3:rd »	8,5	12,5	11,0	5,5	9,4	7,5	10,5	6,5	5,5	7,5
4:th »	9,0	14,0	13,5	6,5	11,8	8,0	11,5	7,5	7,0	8,5
5:th »	9,5	14,0	15,0	7,5	11,5	8,5	10,0	9,5	7,0	8,8
6:th »	10,0	14,5	15,0	8,0	11,9	8,0	11,0	10,0	7,5	9,1
7:th »	12,0	17,0	14,0	9,5	13,1	8,5	12,5	9,0	8,0	9,5
8:th »	12,0	17,5	14,0	10,5	13,5	7,5	14,5	9,0	9,0	10,0
9:th »	12,0	19,5	14,0	10,5	14,0	7,5	15,0	8,0	9,5	10,0
10:th »	13,0	20,0	13,0	11,0	14,3	8,0	15,0	—	—	11,5
11:th »	12,5	21,5	14,5	11,0	14,9	8,5	17,5	—	—	13,0
12:th »	13,0	18,0	11,5	11,5	13,5	7,0	16,5	—	—	11,8

TABLE 2. *The weight of 8 dwarfs (in g.) without a predisposition to adipositas (4 ♂ and 4 ♀), by the end of each of their first 12 months of life.*

	♂ 132	♂ 86	♂ 166	♂ 184	♂ Average	♀ 87	♀ 144	♀ a	♀ 180	♀ Average
1:st month	4,5	7,0	5,0	4,0	5,1	7,0	3,5	4,5	4,5	4,9
2:nd »	5,0	9,5	5,5	4,5	6,1	8,0	4,5	6,0	5,5	6,0
3:rd »	6,5	10,0	7,5	5,0	7,3	9,5	6,5	6,0	6,5	7,1
4:th »	7,5	10,0	7,5	5,0	7,5	9,5	6,0	6,0	7,0	7,1
5:th »	8,5	7,5	8,0	6,0	7,5	9,0	6,5	6,5	7,5	7,4
6:th »	8,5	10,0	9,0	6,5	8,5	9,0	7,0	6,0	7,0	7,3
7:th »	8,0	10,5	9,0	7,5	8,8	9,5	7,0	6,5	7,0	7,5
8:th »	9,0	10,5	8,5	7,5	8,9	9,5	6,5	7,0	8,0	7,8
9:th »	9,0	11,0	8,5	7,5	9,0	10,0	6,5	7,0	8,5	8,0
10:th »	9,5	10,0	8,5	7,5	8,9	8,5	6,5	7,0	7,0	7,3
11:th »	8,5	10,5	8,5	8,0	8,9	9,5	6,5	6,5	7,5	7,5
12:th »	10,5	10,5	9,0	—	10,0	10,0	6,5	7,0	7,5	7,8

The question might be asked whether from human pathology we know any pathologic picture corresponding exactly to the one observed in dwarf-mice with adipositas. It bears a certain outward resemblance to adiposogenital dystrophy, especially when this affection is accom-

panied by dwarfism, or to the LAURENCE—MOON—BIEDL syndrome, which is generally not accompanied by dwarfism, but by anterior pituitary insufficiency. In the case of adiposogenital dystrophy we cannot in the relatives demonstrate isolated occurrence of dwarfism with genital dystrophy or of adipositas. The disease is sometimes found to be familial and may be found in father and son, for which reason some writers, e. g. CLAUSSEN (1939), have supposed a dominant gene to be the main condition for its occurrence. On the other hand HANHART's dwarfism, which clinically greatly resembles adiposogenital dystrophy in its more pronounced forms, shows an undoubted recessive manner of inheritance. The LAURENCE—MOON—BIEDL syndrome is according to ROBERTS (1940) due to a recessive gene; complex explanations based on the variety of the changes are neither necessary nor tenable. But it must be remarked, however, that a few of the relatives of sufferers from the LAURENCE—MOON—BIEDL syndrome display slight abnormalities, particularly obesity and extra digits. This denotes in ROBERTS's opinion that the gene is not truly recessive, but it may of course also be explained otherwise. However, from what we know by now we are not justified in analogizing dwarfism with adipositas in the animals with any of the above-mentioned diseases in man, neither with HANHART's dwarfism, FRÖHLICH's disease, nor LAURENCE—MOON—BIEDL's syndrome.

Fig. 2. Preparation of a mouse with adipositas, showing the large amount of fat deposited in the mesentery.

The combination of adipositas and dwarfism cannot be regarded as an interaction of two truly complementary genes, because each of them manifests itself plainly, when appearing alone. The more recently adopted view that hereditary pathologic conditions are conditioned by monomeric genes is probably correct in most cases. Yet we cannot exclude the possibility that more rarely occurring hereditary diseases (complexes of symptoms or malformations), chiefly such as occur only in siblings, may develop in the

manner that two recessive genes, which have each of them alone a demonstrable effect, meet in one individual.

Conclusions. — (1) Normal hereditary traits are often conditioned by polymeric genes, they being developed by interaction of numerous genes, which taken separately have but little effect. This is, for instance, the case with stature and many other graded characters. Most of the differences between normal persons are differences in graded characters.

(2) Other normal traits depend on a single gene, but naturally also, like all other monomeric traits, on the gene milieu of the whole organism. We do not know many such traits; yet of late years some have been discovered (blood-group traits, secreter or non-secreter traits, power of taste, etc.), and it cannot be excluded, but must even be regarded as likely, that such normal traits are also frequent.

(3) Hereditary diseases are monomeric in many cases, though not in all, but are often also dependent on modifying genes, and always more or less dependent on the entire gene milieu. There are no facts supporting the view formerly prevailing that many of the most common affections, such as deaf-mutism, mongoloid idiocy, schizophrenia, progressive muscular dystrophy, etc. should be dimeric or polymeric.

(4) Predispositions to diseases that in a greater or smaller degree are hereditary must generally be regarded as polymeric. They are graded characters dependent on many genes, which, when appearing alone, have but little effect. This is for instance the case with regard to the predisposition to tuberculosis, and presumably also with regard to the different bodily (constitutional) types, which each of them seem to form the basis of certain mental diseases.

(5) By interaction of several genes, each being pathogenous, there may, however, as shown in the experiments mentioned here, arise pathologic pictures which, when regarded separately, seem to present a unit.

Summary. — By cross-breeding one strain of mice in which there had occurred recessive hereditary dwarfism with another in which there had occurred dominant hereditary adipositas, individuals were produced suffering both from dwarfism and adipositas.

In connection with this observation polymerism in morbid inheritance is discussed.

LITERATURE CITED.

1. BARTELS, E. D. 1941. Studies on hereditary dwarfism in mice. III. — Acta path. scand. XVIII.
2. BAUER, J. and STEIN, C. 1926. Konstitutionspathologie in der Ohrenheilkunde. — Berlin.
3. CLAUSSEN, F. 1939. Ueber asthenische Konstitution. — Z. f. Morph. u. Anthrop. XXXVIII.
4. CSIK, L. and MATHER, K. 1937—38. The sex incidence of certain hereditary traits in man. — Ann. of eugen. VII.
5. CUÉNOT, L. 1905. La loi de MENDEL et l'hérédité de la pigmentation chez les souris. — Arch. Zool. Exp. et Gen.
6. CURTIUS, F. 1933. Multiple Sclerose und Erbanlage. — Leipzig.
7. DAHLBERG, G. 1930. Inzucht bei Polyhybridität beim Menschen. — Hereditas XIV.
8. — 1931. Eine statistische Untersuchung über die Vererbung der Taubstummheit. — Z. f. Konstitutionslehre XV.
9. FOGH-ANDERSEN, P. 1942. Inheritance of harelip and cleft-palate. — Copenhagen. (In the press.)
10. GRUNNET, J. 1942. Studies on hereditary dwarfism in mice. V. — Acta path. scand. (In the press.)
11. HANSEN, K. and v. UBISCH, G. 1927. Der Erbgang der Dystrophia musculorum progressiva. — Deutsche Z. f. Nervenheilk. IC.
12. JUST, G. 1940. Die mendelistischen Grundlagen der Erbbiologie des Menschen. I. — Berlin.
13. KEMP, T. 1933. Hereditary dwarfism in the mouse. — Acta path. scand. Suppl. XVI.
14. — 1938. Heredity and the endocrine function. — Acta path. scand. Suppl. XXXVII.
15. — 1941. Mutation hos Mennesket. — Naturens Verden XXV.
16. KEMP, T. and MARX, L. 1937—38. Beeinflussung von erblichem hypophysärem Zwergwuchs bei Mäusen durch verschiedene Hypophysenauszüge und Thyroxin. — Acta path. scand. XIV und XV.
17. LENZ. F. 1936. BAUR—FISCHER—LENZ: Menschliche Erblehre. I. — München.
18. MACKLIN, M. T. 1929. Mongolian idiocy. The manner of its inheritance. — Am. Journ. of med. sci. N. S. CLXXVIII.
19. MINKOWSKI, M. and SIDLER, A. 1927—1928. Klinische und genealogische Untersuchungen zur Kenntnis der progressiven Muskeldystrophie. — Arch. Klaus-Stiftung III.
20. MØLLENBACH, J. 1941. Studies on hereditary dwarfism in mice. IV. — Acta path. scand. XVIII.
21. PLATE, L. 1913. Vererbungslehre. — Jena.
22. ROBERTS, F. 1940. Medical genetics. — London.
23. RÜDIN, E. 1916. Dementia præcox. — Berlin.
24. SCHULTZ, B. 1940. Kinder schizophrener Elternpaare. — Z. f. Neurologie CLXIX.

25. SJÖVALL, B. 1936. Dystrophia musculorum progressiva. Eine erblichkeitsklinische und medicinische Studie. — Acta psych. neur. scand. Suppl. X.
26. THUMS, K. 1936. Neurologische Zwillingstudien. I. Zur Erbpathologie der multiplen Sclerosen. — Z. f. Neurologie CLV.
27. WEITZE, M. 1941. Hereditary adiposity in mice and the cause of this anomaly. — Copenhagen.

CELLULOSE ACETATE AS A MOUNTING MEDIUM FOR ACETIC SMEARS

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THE ordinary aceto-carmine smear-method for chromosome studies has had a drawback in the fact that it has not been suitable for making permanent preparations. Though various methods have been described for transferring the object into Canada-balsam, all of these are more or less laborious and require dexterity and concentration on the part of the operator. And even then, as has been stated by such experienced cytologists as LA COUR (1937) and GEITLER (1940), in most cases the final preparation will be inferior to the freshly made one. It might be noted, however, that there exist some such methods (HEILBORN, 1937; SVÄRDSON, 1941) by which the preparations reach their final high quality in the permanent stage.

ZIRKLE (1940) has followed quite a different principle to obtain permanent preparations. As he states, the simplest way would be to have a fluid which stains and fixes the object and also contains an ingredient that forms the mounting medium as the other contents evaporate. He has made a systematic search for such fluids and gives several formulae, with which he has had some success. Instead of the difficulties he has avoided, some new ones have arisen, however, e. g. the high viscosity of the mixtures used.

In the following a procedure will be described which is nearly as simple as the ideal set up by ZIRKLE. Though I have not as yet obtained 100 % successes with it, it has in many cases proved possible, with its help, to get permanent preparations which have very much the same appearance as when they were new. (Fig. 1.)

The basic principle of this method is that cellulose acetate is used as a mounting medium. This substance is soluble in acetic acid and can easily be added to any ordinary acetic smear-preparation.

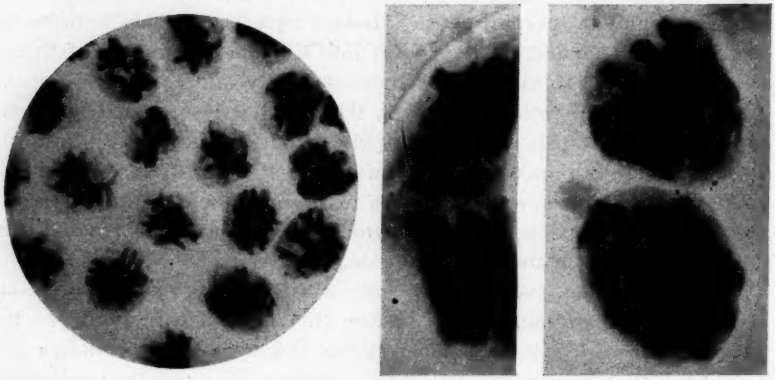
My working schedule for studying the reduction-division in pollen mother-cells of various plants has been the following.

An anther is crushed in a rather small drop of aceto-carmine (or some other suitable acetic stain solution, e. g. the acetic-orcein of LA COUR, 1941), in the middle of a slide. As a tool for this crushing

a piece of glass has been used which has roughly the shape of a spatula, though it is somewhat more bent so that one can easily hold one end while the other end rests against the slide. The tool is made of glass cut from an ordinary microscopic slide.

After the fragments of the anther-wall have been removed with a forceps, a small cover-glass, 18 × 18 mm, is put on the drop. With large cover-glasses the later substitution with cellulose acetate may be more difficult.

Naturally, other manipulations, such as pretreatment for spiral



a *b* *c*

Fig. 1. Aceto-carmine smears of pollen mother-cells mounted in cellulose acetate. — *a—b*: *Paris quadrifolia*. Preparations: May, microphotographs: August, 1942. — *a*: Metaphase I; 300 ×. — *b*: Anaphase II; pretreated with KCN for spiral structure; 1600 ×. — *c*: *Tradescantia virginiana*. Preparation: June, microphotograph: August, 1942. Metaphase I; 1000 ×.

structure or heating and pressing to disentangle prophase threads, may also be made in the ordinary ways. The method has also been applied to root-tip smears (ELVERS, 1942).

The slide is examined in the microscope. If it is found to contain the wanted division-stages, the cellulose acetate is then added.

As material for the cellulose acetate I have used foils of this substance manufactured under the trade name »Briofan» by Albihn & Sandell Co., Gothenburg, Sweden. When the mounting medium is prepared, pieces of these foils are dissolved in glacial acetic acid. (The dissolution requires several hours.) By adding more »Briofan» or more acetic acid the consistence of the mixture can be adjusted so that it will be

about the same as that of Canada-balsam of ordinary viscosity. It should be kept in an airtight jar, so that the acetic acid does not evaporate too freely.

The method for applying this medium is best illustrated by the following sketch (Fig. 2).

The object lies under the cover-glass O. At the edges A and B of this cover-glass the »Briofan» solution is added. It is then covered with auxiliary cover-glasses. The acetic acid would otherwise evaporate and the remaining cellulose acetate would be lying as bone-hard masses, not able to penetrate under the cover-glass O.

When the preparation is manufactured in the right way, the following will happen. The solution under O evaporates slowly at the free edges. The stain is brought along with the solution and precipitates at

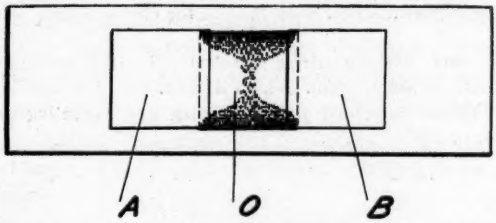


Fig. 2. Explanation in text.

the edges. The evaporating solution is replaced by the cellulose acetate, which is sucked in from A and B. Finally the object will lie mounted in pure cellulose acetate.

The main difficulty met with in the course of preparation is to get enough cellulose acetate in under the central cover-glass. If one does not succeed in that, sooner or later the mounting medium will contract so that irregular vacuoles are formed in it, which are likely to destroy the object. (Such preparations will not necessarily be destroyed, however, as it may happen that the cellulose acetate disappears all around the cells but remains in them and keeps them in good condition.) Other mishaps that may occur are that the stain will remain too long in parts of the preparation, so that the cells there will be overstained, or that some cells will appear shrunken, perhaps owing to the cellulose acetate not having been able to penetrate into these cells as it should have done.

Anyway, a good many of my first-experiment preparations have already been kept for several months without showing any deterioration. (In order to subject them to a severer test they were left lying openly in

a light laboratory.) As the first period, before the stain and the acid have left the preparation, should be the most critical, it seems reasonable to expect that they will remain unchanged also in the future.

September, 1942.

LITERATURE CITED.

1. ELVERS, I. 1942. En kromosom-laboration. — Medlemsblad f. Biologilär. Fören. 8, nr 4 (in the press).
2. GEITLER, L. 1940. Schnellmethoden der Kern- und Chromosomen-Untersuchung. — Berlin.
3. HEILBORN, O. 1937. A new method of making permanent smears with special reference to salivary gland chromosomes of *Drosophila*. — Annals of the Agric. Coll. of Sweden, Vol. 4, p. 89.
4. LA COUR, L. 1937. Improvements in cytological technic. — Bot. Review 5, p. 241.
5. — 1941. Acetic-orcein: a new stain-fixative for chromosomes. — Stain Tech. 16, p. 169.
6. SVÄRDSON, G. 1941. Somatic pairing in *Salmo* and *Coregonus* and its hypothetical explanation. — Ark. f. Zool. Bd. 33 B, nr 9.
7. ZIRKLE, C. 1940. Combined fixing, staining and mounting media. — Stain Tech. 15, p. 139.

GENETICAL EFFECTS OF DUPLICATED FRAGMENT CHROMOSOMES IN RYE

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I. INTRODUCTION.

CHROMOSOME fragments and fragment chromosomes are not only frequently met with in experimental material but also occur in natural populations. Lists of species with such fragments are given by RANCKEN (1934, pp. 60—63) and DARLINGTON (1937, pp. 145—146). In most cases the knowledge of these fragments is limited to their mere occurrence, nothing being known as to their genetical effects. In some species, however, more detailed data are available. A priori the chromosome fragments might be assumed to have similar effects as ordinary chromosomes, though in a lower degree corresponding to their smaller size. This is, indeed, the case, for instance, in the classical cases in *Matthiola* (FROST, 1927) and *Solanum lycopersicum* (LESLEY and LESLEY, 1929).

In contrast to this category, in which the fragments are genetically active, other cases are known in which the fragment chromosomes seem to be inert, having no influence on the visible properties of the individuals. The best example of this kind is *Zea Mays*. In this species supernumerary chromosomes are frequent, as was first observed by LONGLEY (1927) and RANDOLPH (1928 a, b). According to the latter author the extra chromosomes in *Zea* belong to two recognizable types, B chromosomes which are smaller than the smallest standard or A chromosomes, and C chromosomes which are still smaller. Of these the B chromosomes have been best studied, and were found to be genetically inert (RANDOLPH, l. c.; AVDULOW, 1933). As many as twenty-five B chromosomes have been accumulated in one plant by selection without seeming to alter it either in general or in particular ways. MCCLINTOCK (1933) discovered that these chromosomes are heterochromatic in the prophase of meiosis and sometimes at metaphase of mitosis. The properties of the B-chromosomes were further studied in detail by DARLINGTON and UPCOTT (1941). According to these authors the B's are liable to irregularity at mitosis (owing to deficient centromeres) and loss at meiosis (owing to non-pairing). They are also

specially liable to deletion (owing to heterochromatin content), and then give smaller b chromosomes. In spite of this double selection pressure which acts to reduce their size as well as their frequency in the population, they are preserved by an opposite selection in their favour. According to the authors this opposite selection is due to the fact that the B chromosomes, though genetically inert, have important functions in the nucleic acid metabolism of the cell. In this respect they tend to replace the heterochromatic regions of the A chromosomes.

A similar situation may prevail also in other plant species. At any rate, several cases are known in which the supernumerary fragments do not seem to have any genetical effects. Thus, for instance, in *Paspalum stoloniferum* AVDULOW and TITOVA (1934) found an individual with three small extra chromosomes exerting no influence on the phenotype. In the same way a pair of additional fragments in *Sorghum verticilliflorum* had no effect on the morphology and fertility (HUSKINS and SMITH, 1934).

In *Secale cereale* the occurrence and nature of extra chromosomes have been studied for twenty years by quite a number of investigators (cf. the literature cited by MÜNTZING and PRAKKEN, 1941, p. 301. To this enumeration should also be added GOTOH, 1932). In rye populations all over the world occasional plants with 16 instead of 14 chromosomes have been observed. Originally it was assumed (GOTOH, 1924) that the plants with $2n = 16$ have the same amount of chromatin as the plants with $2n = 14$, the higher chromosome number being the result of simple fragmentation. However, as first demonstrated by LEWITSKY (1931) and confirmed by HASEGAWA (1934), the 16-chromosome plants have two fragment chromosomes in addition to the normal set of 14 chromosomes present in ordinary rye plants. Thus, as in *Zea*, the 16-chromosome rye represents a case of duplication of an additional small chromosome. It should be observed, however, that the additional fragments are probably not identical in the different groups of material. In the plants studied by HASEGAWA pairing between the extra chromosomes was very regular. In other Japanese material examined by DARLINGTON (1933) the additional fragment chromosomes were probably smaller. The formation of the fragment bivalent often failed, the fragments frequently being present as univalents. In a second plant with 16 chromosomes also examined by DARLINGTON (l. c., p. 45) the fragments were of unequal size and entirely failed to pair. Thus, 16-chromosome rye does not always mean the same thing.

According to the previous papers on 16-chromosome rye, the extra

chromosomes do not seem to have any noticeable effects on the morphology and fertility of the plants. Thus, HASEGAWA (1934) finds it interesting that this form of rye is quite fertile and that it is found so frequently in most countries where rye is cultivated. DARLINGTON (1937) considers the extra chromosomes in rye to be inert just as in maize and in some other species listed in his Table 16 (l. c., p. 145). On the other hand, POPOFF (1939), studying primitive Bulgarian types of rye, found that plants with extra chromosomes had a decreased percentage of seed setting in comparison with normal plants having $2n = 14$.

II. THE PRESENT MATERIAL.

As described by MÜNTZING and PRAKKEN (1941, p. 278), a single plant with $2n = 16$ was observed in a rye population (of the variety »Östgöta Gråråg») cultivated at Svalöf, Sweden. The two extra chromosomes were distinctly smaller than the ordinary chromosomes and could be recognized at meiosis without difficulty. This was especially easy as in most p. m. c. they appeared as univalents. Counts from three slides gave an average of 71,3 per cent of cells with unpaired fragment chromosomes. In the remaining 28,7 per cent of the cells the fragments formed a small bivalent. Pairing between the fragments and the ordinary chromosomes was not observed.

In order to undertake a closer study of the cytology and genetics of rye plants with extra fragment chromosomes a progeny was raised from the original plant with 16 chromosomes. This progeny was derived from seeds obtained after open pollination. Counts of the somatic chromosome numbers in twenty of the plants gave the following result: Ten plants had $2n = 14$, two plants $2n = 15$, and 8 plants $2n = 16$ [1]. This result was rather unexpected, as the mother plant had been openpollinated and was surrounded by plants having $2n = 14$. As most rye plants are highly self-sterile, the majority of the daughter plants in this case would be expected to have $2n = 15$. The remaining plants should have $2n = 14$, due to elimination of the fragment chromosomes at meiosis in the mother plant. The same peculiar excess of plants with $2n = 16$ and deficit of plants with $2n = 15$ was observed by LEWITSKY, MELNIKOV and TITOVA (1932) in quite analogous crosses

[1] Here and in the following text the plants with two extra chromosomes are said to have $2n = 16$ though $2n = 14 + 2\,f$ would be a more precise denomination. The same simplification applies to the plants with other numbers of extra fragment chromosomes.

between rye plants with 2n = 14 and 2n = 16 (both reciprocal combinations). The probable cytological cause of these aberrant ratios was observed by HASEGAWA (1934). According to this author the extra chromosome shows a rather irregular behaviour at anaphase of the primary nuclear division in the pollen grain. The two split halves of this fragment chromosome often do not separate, and in most cases they are included in the generative nucleus. As in our case, and in the cross 16 ♀ × 14 ♂ studied by LEWITSKY and his co-workers, the same aberrant ratios were obtained when the 16-chromosome plant was the female parent, similar processes of non-disjunction as in the pollen may be assumed to occur during the development of the female gametophyte.

At any rate a number of daughter plants with 2n = 16 were obtained. These plants were intercrossed in the summer of 1940, and from the seeds obtained in this way a total of 224 plants were raised from ten different mother plants. The somatic chromosome numbers of all these plants were determined, the total frequency being the following:

Chromosome number:	14	15	16	17	18	19	20
Number of plants: ..	77	27	77	10	32	—	1

The chromosomal distribution in the separate progenies may be considered later on. Now it is sufficient to report that each of the ten progenies comprised plants with 2n = 14 as well as plants with different numbers of extra fragment chromosomes. These plants were distributed at random in the rows. The kernels had been sown in small pots. After fixation, but before the chromosome numbers were known, the plants were put in a hotbed. They wintered very well and only eight plants died before the next summer. Evidently this material was quite suitable for studies of the genetical effects of the extra fragments present in more than half of the plants. Therefore, in order to study the viability and fertility of the plants the following properties were measured and calculated: plant weight, kernel weight, straw weight, plant height, number of culms, number of seeds per spike, percentage of good pollen.

A new generation of this material was grown in 1942. These plants were derived from crosses or isolations of mother plants with a variable number of extra fragments. As in 1941, each progeny comprised plants with 2n = 14 besides plants with extra fragment chromosomes. These

plants were measured in the same way as before, and in this year also the percentage of seed setting was determined. At present the material obtained after isolation will not be considered, as it is not comparable with the plants raised from crosses in the preceding year. However, about half of the plants studied in 1942 were derived from open-pollinated or artificially crossed mother plants. These plants, amounting to 92 individuals, are of quite the same kind as those studied in 1941. The results of the measurements gathered from the two sets of material were also quite similar. This is evident from the tables and figures discussed below.

In the present report only the genetical effects of the extra chromosomes will be considered. The cytological side of the question will be treated in more detail in another paper. It should be mentioned already now, however, that the extra chromosomes in my material, just as in the plants studied by LEWITSKY and HASEGAWA, represent a special type of chromosome not present in ordinary rye plants with $2n = 14$. In contrast to the normal chromosomes, which are more or less V-shaped, the extra chromosomes have a subterminal centromere. By this character and by their small size the fragment chromosomes may easily be distinguished also in somatic plates. Meiosis was studied preliminarily in some daughter plants of the primary plant with 16 chromosomes. In these plants the fragment chromosomes showed essentially the same behaviour as described for the mother plant (MÜNTZING and PRAKKEN, 1941, pp. 278—279). The fragments are frequently unpaired and do not seem to pair with the major chromosomes either. Otherwise, meiosis was found to be quite regular, even in plants with four extra chromosomes.

III. THE RESULTS OF THE MEASUREMENTS.

1. VEGETATIVE DEVELOPMENT.

In the first place plant vigour was determined by measurements of *plant weight* after the harvest (the root system being excluded). The results are given in Table 1. In order to save space the data from both years are included in the same table. As the separate progenies in each year were found to behave in the same way, their values were added to total series.

In 1941 the following average values [1] were obtained (the number

[1] Here and in the sequel only average values based on at least five variates are mentioned in the text.

TABLE 1. *Correlation between plant weight and number of fragments.*

Year	Number of fragments	Plant weight 0	10	20	30	40	50	60	70	80	90	100	110 gr.	n	$\bar{X}$ (in gr.)
1941	0	9	28	21	9	7	1	1						76	22,90
1942	»		2	4	5	—	1	2	—	—	—	1		15	39,67
1941	1	2	9	11	2	—	—	1						25	22,20
1942	»		1	—	1									2	(25,00)
1941	2	12	24	28	6	2	—	—	1					73	20,48
1942	»	3	7	9	15	6	1	1	2					44	32,05
1941	3	2	6	1	1									10	16,00
1942	»		1	1										2	(20,00)
1941	4	11	18	2										31	12,10
1942	»	2	4	6	4	2	1	—	—	1				20	29,50
»	5		1	—	1									2	(25,00)
1941	6		1											1	(15,00)
1942	»	1	5											6	13,33
»	8	1												1	(5,00)

of fragments in brackets): 22,90 (0); 22,20 (1); 20,48 (2); 16,00 (3); 12,10 (4). Evidently *there is a negative correlation between plant weight and the number of fragment chromosomes present.* The significance of this correlation was tested with the analysis of variance. The result of this calculation was as follows:

	Degrees of freedom	Sums of square	Mean square
Between series:	5	28,9406	5,7881
Within » :	210	269,0409	1,2811
Total:	215	297,9815	—

The value of v^2 (using the terminology and tables of BONNIER and TEDIN, 1940), will be 4,5181, which corresponds to a P smaller than 0,001. Thus, it is quite certain that the presence of extra fragment chromosomes causes a decrease in plant weight. This decrease, however, is most marked in respect of the higher numbers of fragments. If the standard errors of the means are calculated on basis of the mean square for »within series» (1,2811), the average values and standard errors for the series 0, 2 and 4 fragments will be $22{,}90 \pm 1{,}30$; $20{,}48 \pm 1{,}32$ and $12{,}10 \pm 2{,}03$. The difference between the first two values, $2{,}42 \pm 1{,}36$, is not significant, the t-value only amounting to 1,30. The difference between the 2- and 4-series, on the other hand, will be $8{,}38 \pm 2{,}43$, which corresponds to a t of 3,449 and a P of about 0,001.

The data from 1942 show a quite similar negative correlation between plant weight and number of fragments, the following average values being obtained (Table 1): 39,67 (0); 32,05 (2); 29,50 (4); 13,33 (6). This year the environmental conditions were more favourable for plant development than in 1941. Therefore the average values are higher, and even the plants with 4 extra fragments had a rather good vigour. However, when the number of fragments is as high as 6 there is again a very pronounced decrease in weight. A calculation of v^2 from an analysis of variance of this material gave the value 1,904, which corresponds to a P somewhat larger than 0,05. A smaller P would certainly

TABLE 2. *Correlation between kernel weight and number of fragments.*

Year	Number of fragments	Kernel weight per plant													n	$\bar{X}$ (in gr.)
		0,0	2,5	5,0	7,5	10,0	12,5	15,0	17,5	20,0	22,5	25,0	27,5	30,0 gr.		
1941	0	8	14	13	17	8	3	5	4	2	1	1			76	8,85
1942	»		2	6	1	2	2	—	1	—	—	—	1		15	10,08
1941	1	1	4	7	4	6	2								25	8,65
1942	»			1	—	1									2	(8,75)
1941	2	18	17	20	10	7	—	—	—	—	—	1			73	5,53
1942	»	8	10	10	7	4	2	2	—	1					44	6,75
1941	3	3	2	4	—	1									10	4,75
1942	»	2													2	(1,25)
1941	4	21	6	2	1										30	2,33
1942	»	9	6	3	1	—	1								20	3,75
»	5	1	1												2	(2,50)
1941	6	1													1	(1,25)
1942	»	6													6	1,25
»	8	1													1	(1,25)

have been obtained if the number of plants available this year had been greater. Anyhow, the values of 1942 point in just the same direction as the significant data of the preceding year.

As plant weight is the sum of kernel and straw weight it seemed desirable to test the latter two properties separately. The correlation between number of fragments and *kernel weight* is represented in Table 2. A glance at the table is sufficient to reveal the presence of a rather marked negative correlation. — In 1941 the following average values were obtained (the number of fragments in brackets): 8,85 (0); 8,65 (1); 5,53 (2); 4,75 (3) and 2,33 (4). The significance of this decrease was tested with the analysis of variance, the following result being obtained:

	Degrees of freedom	Sums of square	Mean square
Between series:	5	189,0421	37,8084
Within » :	209	716,5672	3,4286
Total:	214	905,6093	—

This gives a v^2-value of 11,0273 and a P much smaller than 0,001. This result was also completely verified by the values obtained in 1942. In this year the classes with different numbers of extra fragments had the following average values: 10,08 (0); 6,75 (2); 3,75 (4) and 1,25 (6). This decrease is also significant, as was proved by an analysis of

TABLE 3. *Correlation between straw weight and number of fragments.*

Year	Number of fragments	Straw weight 0	10	20	30	40	50	60	70	80 gr.	n	$\bar{X}$ (in gr.)
1941	0	25	35	14	2						76	14,08
1942	»		6	5	—	2	1	—		1	15	29,00
1941	1	5	19	—	1						25	13,80
1942	»	1	—	1							2	(15,00)
1941	2	17	43	10	2	1					73	15,00
1942	»	3	13	13	11	2	2				44	25,46
1941	3	6	3	1							10	10,00
1942	»		1	1							2	(20,00)
1941	4	14	16	1							31	10,81
1942	»	2	5	7	2	3	—	—		1	20	27,00
»	5		1	—	1						2	(25,00)
1941	6		1								1	(15,00)
1942	»	1	5								6	13,33
»	8	1									1	(5,00)

variance. In this case the mean square for »between series» was found to be 14,1536 and for »within series» 3,3737. This gives a v^2 of 4,1953 and a P of approximately 0,001.

The negative correlation between number of fragments and total plant weight is evidently less pronounced than the correlation with kernel weight. This being the case it is of interest to study the possible correlation between fragment number and *straw weight*. This was done with the above result (Table 3). From the distribution of the variates it is evident that the correlation is very weak, if there is any correlation at all. In 1941 the average values were the following: 14,08 (0); 13,80 (1); 15,00 (2); 10,00 (3) and 10,81 (4). The two last values

are the lowest ones, indicating a negative correlation, but the plants with 2 fragments had a higher straw weight than those without fragments. An analysis of variance gave the following figures.

	Degrees of freedom	Sums of square	Mean square
Between series:	5	5,3267	1,0653
Within » :	210	113,0437	0,5383
Total:	215	118,3704	—

The v^2-value (1,0653 : 0,5383) will be 1,9790, which corresponds to a P situated between 0,05 and 0,20. According to these values there is no significant heterogeneity in the material and hence no significant correlation between number of fragments and straw weight. However, in spite of this result it is rather probable that the plants with the highest numbers of fragments are really less vigorous, i. e. have a lower straw weight. This may be studied by a calculation of the standard errors from the mean square of »within series». If this is done the $\bar{X} \pm m$ values of the 0-, 2- and 4-series will be $14{,}08 \pm 0{,}84$, $15{,}00 \pm 0{,}86$ and $10{,}81 \pm 1{,}32$ respectively. Evidently the difference between the first two values is not significant. The difference 14,08 — 10,81, on the other hand, will be $3{,}27 \pm 1{,}57$, giving a t of 2,085 and a P situated between 0,05 and 0,02 (105 degrees of freedom). The difference 15,00 — 10,81, finally, will be $4{,}19 \pm 1{,}58$. This gives a t of 2,654, the corresponding P (for 102 degrees of freedom) being approximately 0,01. Thus, these data strongly indicate that the plants with 4 fragments really have a lower straw weight than those with 0 or 2 fragments.

The data from 1942 gave a similar result, the following average values being obtained (Table 3): 29,00 (0); 25,46 (2); 27,00 (4); 13,33 (6). In this year, which favoured the vegetative development, the plants with 4 fragments had about the same straw weight as those with 0 and 2 fragments. The plants with 6 fragments, however, seem to be inferior. An analysis of variance of this material gave a v^2 of only 1,3136 and a P larger than 0,2. However, if the standard errors are calculated in the usual way, the difference between the most extreme values 29,00 (0 fragments) and 13,33 (6 fragments) will be $15{,}67 \pm 6{,}75$. This will give a t of 2,321 and a P (for 19 degrees of freedom) intermediate between 0,02 and 0,05.

Thus, considering all data from both years, it may be concluded that in the plants with the maximum number of fragments straw weight is lower than in the other plants.

As this fact could only be demonstrated with some difficulty, it is desirable also to consider some other kinds of measurements of the vegetative development, viz. plant height and number of culms. From want of space the distributions cannot be published but only the average values and the results of analyses of variance. The following values were obtained in 1941 and 1942:

Number of fragments	1941 Plant height	1941 n	1942 Plant height	1942 n
0	124,48	76	129,00	15
1	125,90	25	(135,00)	2
2	120,10	73	128,41	44
3	127,00	10	(130,00)	2
4	113,30	31	127,50	20
5	—	—	(125,00)	2
6	(122,50)	1	110,00	6
8	—	—	(85,00)	1

Considering first the values of 1941, the occurrence of a significant heterogeneity was demonstrated by an analysis of variance. The mean squares for »between» and »within» series were found to be 29,4178 and 5,6234 respectively, giving a v^2 of 5,2313 and a P smaller than 0,001. In this case it is of value to divide the sum of squares into two parts, one corresponding to the variation between the 0-series and the sum of the other series, the other to the variation between the other series. This gives the following result:

Cause of variation	Degrees of freedom	Sums of square	Mean square
Between the 0-series and the sum of the other series	1	36,9563	36,9563
Between the 1- to 8-series	4	110,1331	27,5332
Within series	210	1180,9106	5,6234
	Total 215	1328,0000	—

The quotient 36,9563 : 5,6234 will be 6,572. This value of v^2 corresponds to a P only slightly larger than 0,01. Consequently, it is highly probable that, on an average, plant height is lower in plants with fragments than in plants which are quite free from fragments. This conclusion is supported by the additional data from 1942. The average values of

this year just given represent a clearly decreasing series. The heterogeneity of this material from 1942 was again tested with the analysis of variance. The mean squares for »between» and »within» series were found to be 5,4286 and 2,3002 respectively. This gives a v^2 of 2,3601 and a P intermediate between 0,05 and 0,01. — Thus, the results of both years combined leave no reasonable doubt as to the occurrence of a weak negative correlation between plant height and number of fragments.

A similar result was also observed with respect to the *number of culms* per plant. The following average values were obtained.

	1941		1942	
Number of fragments	Number of culms	n	Number of culms	n
0	6,79	76	11,80	15
1	7,16	25	(8,00)	2
2	7,60	73	9,68	44
3	6,20	10	(9,00)	2
4	5,71	31	10,20	20
5	—	—	(10,00)	2
6	(5,00)	1	4,00	6
8	—	—	(5,00)	1

The analysis of variance of the 1941 series gave the mean squares 4,5521 and 2,2755 for »between» and »within» respectively. This gives a v^2 of 2,001 and a P intermediate between 0,05 and 0,20. The same treatment of the 1942 material gave the mean squares 10,5383 and 4,4617 for »between» and »within» respectively. This corresponds to $v^2 = 2{,}362$ and P situated between 0,01 and 0,05. Thus, the occurrence of a heterogeneity is indicated rather strongly but is not definitely proved. However, it may be correct in this case to compare the extreme series in each year, i. e. the 0- and 4-series in 1941 and the 0- and 6-series in 1942. This was done in the usual way. For the 1941 material this comparison resulted in a v^2 value of 8,0660 and a P intermediate between 0,001 and 0,01. The 1942 series gave a v^2 of 12,013 and a P which again is intermediate between 0,001 and 0,01. Thus, there can be no doubt that a high number of fragments tends to reduce the number of culms.

Taking all data together concerning the influence of the fragment chromosomes on the vegetative development, we find a slight but significant effect regarding straw weight as well as plant height and number of culms. In all cases, however, the effect is only obvious when a high number of fragments are present. One or two fragments

may have an effect, but, at any rate, this effect is rather slight. Not until the number of fragments amounts to 4 or more is a deleterious influence conspicuous. This deleterious action was more pronounced in 1941 than in 1942, the average plant development in the former year being less good than in the latter.

2. FERTILITY.

As already reported, kernel weight per plant was found to show a marked negative correlation with the number of fragments. This indicates that the degree of fertility is more influenced by the fragment chromosomes than the vegetative development. This was, indeed, found to be true, as is evident from the following data. The *number of kernels per ear* was calculated in 1941 and 1942, the following results being obtained:

Number of fragments	1941 Number of kernels per ear	1941 n	1942 Number of kernels per ear	1942 n
0	42,37	76	32,33	15
1	43,00	25	(35,00)	2
2	32,95	73	24,09	44
3	28,00	10	(5,00)	2
4	22,00	30	10,50	20
5	—	—	(10,00)	2
6	(5,00)	1	5,00	6
8	—	—	(5,00)	1

In both years the average numbers very clearly decrease as the number of fragment chromosomes increases. The significance of this decrease was tested with the analysis of variance and for both years the P values obtained were much smaller than 0,001. Stating the data more precisely, the 1941-series gave the mean squares 24,8551 and 1,1725 for »between» and »within» series respectively. The corresponding values of the 1942-material were 10,6727 and 0,8574. Thus, the two v^2 values will be 21,198 and 12,448 respectively.

As regards the data of 1941 the standard errors were also calculated in the usual way and a number of differences between separate series tested. The results may be summarized as follows:

Difference tested	Degrees of freedom	t	P
0—1 = — 0,632 ± 2,50	99	—	—
0—2 = + 9,423 ± 1,78	147	5,294	< 0,001
0—3 = + 14,368 ± 4,05	84	3,548	»
0—4 = + 20,368 ± 2,34	104	8,704	»
0—6 = + 37,368 ± 10,90	75	3,428	»
2—4 = + 10,945 ± 2,35	101	4,657	»

According to these values the difference between the 0- and 1-series is not significant, in contrast to all the other differences between the series without fragments and the series having from 2 to 6 fragments. The difference between the 2- and 4-series is also significant.

In order to elucidate the correlation between number of fragments and fertility on the female side the *percentage of seed setting* was also calculated, i. e. the number of kernels per 100 flowers. Data of this kind are only available from the 1942-material. As expected, the negative correlation between number of fragments and percentage of seed setting was found to be very pronounced, as is evident from the following average values:

Number of fragments	Number of plants	Average percentage of seed setting
0	15	61,67
1	2	(80,00)
2	44	51,91
3	2	(25,00)
4	20	29,50
5	2	(10,00)
6	6	13,33
8	1	(5,00)

As the decrease is very marked, it is superfluous to test the statistical significance of the data. Moreover, the occurrence of a rather strong negative correlation between number of extra fragment chromosomes and seed setting has already been demonstrated by the previous analysis of kernel weight per plant and number of kernels per ear.

It remains, however, to consider the influence of the fragment chromosomes on *pollen fertility*. — The percentage of good pollen of all the plants available was estimated in both years, using classes with a class breadth of 10 per cent. In 1941 the following average values were obtained: 91,0 (0); 90,6 (1); 87,2 (2); 75,0 (3) and 66,3 (4). The decrease

is rather obvious. A similar result was obtained in 1942, as is evident from the following average values: 91,0 (0); 89,3 (2); 83,0 (4) and 77,0 (6). As the values of both years are quite similar they were combined to one table (Table 4), which also includes some classes not yet mentioned with less than 5 variates. As three of the series in Table 4

TABLE 4. *Correlation between pollen fertility and number of fragments.*

Number of fragments	Per cent good pollen							n	$\bar{x}$
	40	50	60	70	80	90	100		
0				2	4	22	62	90	91,00
1					1	9	17	27	90,93
2			2	3	12	36	57	110	88,00
3				4	3	3	1	11	75,91
4		3	4	7	14	10	5	43	74,07
5					1	—	1	2	(85,00)
6					5	1		6	76,67
8		1						1	(45,00)

are very skew (the 0-, 1- and 2-series), the significance of the decrease in pollen fertility with an increasing number of fragments was not tested with the analysis of variance. Instead, the χ^2 method was used. The calculations were simplified by combining the variates into the following two series:

	Percentage of good pollen				
	40 — 70	— 80	— 90	— 100	n
0 fragments:	2	4	22	62	90
From 1 to 8 fragments: ..	24	36	59	81	200

A calculation of χ^2 in this case gave the value 25,60. As there are 3 degrees of freedom, the corresponding P will be much smaller than 0,001. Thus, it is quite certain that the presence of extra fragments tends to decrease pollen fertility. The decreasing series of average values also shows that the degree of fertility reduction is roughly proportional to the number of fragments.

When the pollen samples were being collected, it was observed that some plants had more or less defective anthers which did not shed any pollen. For that reason a total of 21 plants are not represented in Table 4. Of these 21 plants only 2 belong to the 0-series, 1 had one fragment, 8 had two fragments, 1 had three fragments and 9 had four

fragments. As 90 of the 290 plants in Table 4 have 0 fragments, the expected value per 21 would be 6,5172. In the same way the expected number of plants with fragments among the 21 exceptional plants would be 14,4828. The difference between the distributions 2 : 19 and 6,5172 : 14,4828 was tested with the χ^2 method. The χ^2 in this case was found to be 4,5399, which gives a P intermediate between 0,02 and 0,05. Thus, it is rather probable that plants with extra fragments have a stronger tendency to form defective anthers than plants without fragments. This conclusion is further supported by the fact that the difference between »observed» and »expected» was most pronounced in the 4-series with the highest number of fragments, the values being 9 and 3,1138 respectively.

IV. DISCUSSION.

The measurements undertaken in my material demonstrate that the extra fragment chromosomes influence plant vigour as well as fertility. The effect of the fragments, however, is relatively slight, and the negative correlation between number of fragments and vegetative development could only be demonstrated by a detailed statistical treatment of the data. The effect on fertility, on the other hand, is more pronounced. This effect was also noticed by POPOFF (1939). In one group of material (l. c., Table 3) he determined the percentage of seed setting in two normal plants and in seven plants with 15 to 18 chromosomes. In the former plants the percentage of seed setting was 78,6, in the latter plants it ranged from 69,3 to 20,9. In another group of material (l. c., Table 4) 8 normal plants had a percentage of seed setting of 79,3, the corresponding values of 32 plants with 1, 2 or 4 extra chromosomes being 54,7, 46,1 and 13,4 respectively. As regards the pollen, POPOFF mentions that plants with 15 and 16 chromosomes have almost the same good pollen as normal plants. In plants with 18 chromosomes he found more pronounced disturbances. Thus, it is clear that in my material as well as in POPOFF's the fragment chromosomes are not inert. It is probable that the same result will be obtained by closer studies of 16-chromosome plants in other rye populations as well.

In *Solanum lycopersicum* LESLEY and LESLEY (1929) observed a non-linear relation between fragment number and fruit size, the potency of two additional fragments in diminishing fruit size being nearly three times that of one such fragment. Similar curved regression lines are

evidently typical of the present data, especially as regards the correlation between number of fragments and vegetative development. A decrease in straw weight, plant height and number of culms could only be demonstrated for the series having the highest number of fragments. With regard to fertility the presence of two fragments causes a slight but significant decrease and also plants with only one fragment are probably somewhat less fertile than normal plants. Quite pronounced decreases, however, are only obtained in the presence of four or more fragments, plants with four fragments being much more than twice as sterile as those with two fragments. This non-linear relationship is quite evident from all the data on kernel weight per plant, number of kernels per ear, percentage of seed setting, and pollen fertility.

Though the fragment chromosomes studied are not inert it is possible that they are *sub-inert*, i. e. have a lower proportion of active genes than ordinary chromosomes. This suspicion is strengthened by some information available on trisomic rye plants. Two years ago measurements were made on 18 daughter plants of a triploid rye individual. Of these plants eight had $2n = 14$, eight $2n = 15$, and two plants $2n = 16$. Plant weight, kernel weight and straw weight as well as plant height and number of kernels per ear were measured. The following results were obtained:

Chromosome number	14	15	16
Average plant weight:	15,00 gr.	5,00 gr.	2,50 gr.
» kernel » :	3,50 »	0,75 »	0,50 »
» straw » :	11,50 »	4,25 »	2,00 »
» plant height:	92,5 cm.	65,6 cm.	52,5 cm.
» number of kernels per plant:	100,0	31,3	12,5

Evidently, the plants with extra chromosomes are in all respects by far inferior to the normal plants with $2n = 14$.

The fragment chromosome in our material has about half the size of an ordinary chromosome, but judging from the above data it has much less than half the effect of an ordinary chromosome. Plants with two extra fragments only differ very slightly from normal plants, whereas the trisomic plants under consideration show a violent reaction against the extra chromosome present. A trisomic plant observed by TAKAGI (1935) was a slender dwarf but had rather good fertility. One

of the trisomic plants studied by MÜNTZING and PRAKKEN (1941, p. 275), on the other hand, had quite good vigour as well as fertility. More data on the properties of trisomic rye plants are needed before any safe conclusions can be drawn. However, the evidence already available supports the view that the fragment chromosomes under consideration are sub-inert.

From the data given in the present paper it is clear that the plants with extra fragments are inferior in several respects to the plants without fragments. Under such circumstances they should disappear rather rapidly from the populations. Nevertheless, plants with 16 chromosomes have been reported repeatedly to occur in the rye populations of widely different parts of the world. This occurrence needs a special explanation. POPOFF (1939) calls attention to the fact that a certain proportion of the kernels are lost at the harvest (especially in fields in which wheat and rye are in a mixed culture) and that the proportion of lost seeds is higher in the fertile plants than in the partially sterile plants having extra chromosomes. By special tests he found a kernel loss of 22,4 % in the fertile plants and 11,3 % in the partially sterile ones. In this way the partially sterile plants with extra chromosomes would escape elimination. I doubt, however, that this differential loss is sufficient to explain the whole story. In my material the average kernel weight in the plants with 14 and 16 chromosomes was found to be 8,85 and 5,53 in 1941 and 10,08 and 6,75 in 1942. Thus, in 1941, seed production per plant in the 16-chromosome category was 38 per cent lower than among the plants with $2n = 14$, the corresponding figure of 1942 being 33 per cent. Even if the 14-chromosome plants should lose 22,4 per cent of their kernels and the 16-chromosome plants no kernels at all, the latter category would nevertheless be eliminated rather rapidly. Moreover, under Swedish conditions of cultivation the kernel losses are probably less than twenty per cent, the plants being less overripe at the time of harvest.

To explain the frequent occurrence of B-type chromosomes in maize DARLINGTON and UPCOTT (1941) assume that these genetically inert chromosomes have important functions in the nucleic acid metabolism of the cell. It is possible that the presumably sub-inert fragment chromosomes in rye may have similar functions. So far, however, it is not known whether these fragments are heterochromatic or not. Attempts to settle this question will be made with the help of the new and promising »nucleic acid starvation» technique devised by DARLINGTON and LA COUR (1940—1941). The suitability of rye

for such experiments has already been demonstrated by LEVAN (1942).

Even if the fragment chromosomes in rye should turn out to be heterochromatic, it is difficult to see how the possible advantages of this heterochromatic constitution would be able to secure the maintenance of plants with 16 chromosomes in the population. The fact remains that in the present material the plants with 16 chromosomes had a kernel production which was more than 30 per cent lower than that of the normal plants with $2n = 14$. Under such circumstances the only explanation possible seems to be that 16-chromosome strains in the rye populations are shortlived, but are frequently produced anew from plants with 14 chromosomes. This frequent production of new 16-chromosome strains would explain that such plants have repeatedly been observed before the strains are extinguished. This hypothesis is supported by the following facts: As pointed out in the introduction there are obvious differences between the 16-chromosome strains so far known in rye, the chromosomes involved showing different degrees of pairing and probably representing different sizes. This was also clearly the case in my own material. The supernumerary fragment chromosomes discussed in the present paper were found in the variety »Östgöta Gråråg» and were characterized by a relatively small size and a frequent non-conjunction at meiosis. Two years ago I found another plant with 16 chromosomes in the variety »Vasa II». According to preliminary observations the extra chromosomes in this case are much larger than in the other strain and show a regular pairing. Progenies have been raised from this new plant, including hybrids with plants carrying the small fragment chromosome of »Östgöta Gråråg». Thus, there will be an opportunity to study the behaviour of extra chromosomes of different size and origin in the same plants.

The repeated production of rye plants with small extra chromosomes is much easier to understand now, since it has been shown that fragmentation and other structural alterations do not only occur in inbred rye (LAMM, 1936; PRAKKEN and MÜNTZING, unpublished data) but are also frequently met with in population plants of rye (MÜNTZING and PRAKKEN, 1941). Fragmentation may occur in ordinary plants with $2n = 14$ (l. c., Figs. 1—2), and fragments may also be produced by inversion heterozygotes (centric fragments by rupture of the dicentric chromatids). Finally, the spontaneous production of tetraploid plants or chimaeras (cf. l. c., p. 294) may also lead to the formation of new chromosome fragments. This may happen because the tetraploids

will give rise to triploids, and the triploids to trisomic plants (which have been observed to occur in the populations, l. c., p. 275). It is a well-known fact that new chromosome types are frequently produced by crossing over in triploids and trisomics.

Thus, it is not difficult to understand that new chromosome fragments may arise in rye populations. They are just one of the several symptoms of the cytological lack of balance which is characteristic of rye and probably also of other allogamous species. Following fragmentation the first deviating plants will probably have one extra fragment chromosome. As the presence of such a fragment interferes very little with plant vigour and fertility, a plant with one fragment will easily produce offspring. Owing to the peculiar chromatid non-disjunction of the fragment at the first division in the pollen grain (HASEGAWA, 1934) many pollen grains will carry two fragments and give rise to plants with two extra fragment chromosomes in the next generation. The same phenomenon also seems to occur on the female side, as was pointed out above (p. 94). Thus, it is easy to understand that 16-chromosome plants may arise in the offspring of plants with single extra fragments (having a centromere). In the offspring of 16-chromosome plants individuals with a still higher number of fragments may be formed but will be rapidly eliminated on account of their poor fertility and reduced vigour. The 16-chromosome plants themselves will soon disappear as well, but are now and then replaced by individuals having new types of duplicated fragments.

POPOFF (1939) assumes that the extra chromosomes observed by him in Bulgarian rye populations owe their origin to spontaneous crosses with the wild related species *Secale montanum*. This explanation is impossible for the present cases of 16-chromosome rye, as *S. montanum* is not growing wild in Sweden. The assumption of a species cross as the ultimate cause of the presence of duplicated extra chromosomes is also made by CAMERON (1934) with regard to *Crepis syriaca*. In this species the normal chromosome number is $2n = 10$, but very often a small characteristic supernumerary unit is present one or several times. This chromosome seems to have only a weak effect when present one, two, or even three times, but individuals with five or more supernumeraries are entirely sterile and those with fewer are partly so. Abnormal flower-head development is regularly associated with the presence of two or more extra units. Plants with 10 and 11 chromosomes were very similar in appearance, but higher-numbered individuals varied considerably from the normal. From these

statements of CAMERON it is evident that the fragments in *Crepis syriaca* are comparable with those in rye. They have a slight but obvious effect and seem to be sub-inert as in rye. In some way, however, they may be favourable to the species, as the accessions from the wild always showed many individuals with extra chromosomes.

In my rye material more information as to the origin and constitution of the extra fragment chromosomes may be gathered from further cytological studies, which are now in progress, and from experimental work with the two types of extra fragment chromosomes available.

V. SUMMARY.

1. In the offspring of a rye plant with two extra fragment chromosomes a material was raised with the number of fragments ranging from 0 to 8. The genetical effects of the fragment chromosomes were studied in two years on a total of 308 plants.

2. Taking all data together concerning the influence of the fragment chromosomes on the vegetative development, a slight but significant effect could be demonstrated regarding straw weight, as well as plant height and number of culms. In all cases, however, the effect was only obvious when a high number of fragments were present.

3. Fertility was found to be more influenced by the fragment chromosomes than the vegetative development. There was a marked and significant, though non-linear, negative correlation between number of fragments and the following properties: kernel weight per plant, number of kernels per ear, percentage of seed setting, and pollen fertility.

4. The measurements demonstrate that the duplicated fragment chromosomes are not inert, as was supposed by some previous workers. However, considering the size of the fragments and the effects of whole extra chromosomes, it is probable that the fragments are sub-inert, having a lower proportion of active genes than ordinary chromosomes.

5. The origin of rye plants with duplicated fragment chromosomes is discussed. It is concluded that 16-chromosome strains in rye populations are shortlived, but that they are frequently produced anew from plants with 14 chromosomes. This is possible on account of the rather frequent occurrence in rye populations of fragmentation and other structural and numerical chromosome alterations.

LITERATURE CITED.

1. AVDULOW, N. 1933. On the additional chromosomes in maize. — Bull. Appl. Bot., Genet. a. Plant-Br., Series 2, No. 2, pp. 101—130.
2. AVDULOW, N. and TITOVA, N. 1934. Additional chromosomes in *Paspalum stoloniferum* BOSCO. — Bull. Appl. Bot., Genet. a. Plant-Br., pp. 165—172.
3. BONNIER, G. och TEDIN, O. 1940. Biologisk variationsanalys. — Stockholm.
4. CAMERON, D. R. 1934. The chromosomes and relationship of *Crepis syriaca* (BORNM.). — Univ. Calif. Publ. Agricult. Sc., Vol. 6, pp. 257—286.
5. DARLINGTON, C. D. 1933. The origin and behaviour of chiasmata. VIII. *Secale cereale* (n., 8). — Cytologia, Vol. 4, pp. 444—452.
6. — 1937. Recent advances in cytology. Second edition. — London.
7. DARLINGTON, C. D. and LA COUR, L. 1940. Nucleic acid starvation of chromosomes in *Trillium*. — Journ. of Genetics, Vol. 40, pp. 185—213.
8. — and —. 1941. The detection of inert genes. — Journ. of Heredity, Vol. 32, pp. 115—121.
9. DARLINGTON, C. D. and UPCOTT, M. B. 1941. The activity of inert chromosomes in *Zea Mays*. — Journ. of Genetics, Vol. 41, pp. 275—296.
10. FROST, H. B. 1927. Chromosome-mutant types in stocks (*Matthiola incana* R. BR.). I. Characters due to extra chromosomes. — Journ. of Heredity, Vol. 18, pp. 475—486.
11. GOTOH, K. 1924. Über die Chromosomenzahl von *Secale cereale* L. — Bot. Mag. (Tokyo), Vol. 38, pp. 135—152.
12. — 1932. Further investigation on the chromosome number of *Secale cereale* L. — Jap. Journ. of Genet., Vol. 7, pp. 172—182.
13. HASEGAWA, N. 1934. A cytological study on 8-chromosome rye. — Cytologia, Vol. 6, pp. 68—77.
14. HUSKINS, C. L. and SMITH, S. G. 1934. A cytological study of the genus *Sorghum* PERS. II. — Journ. of Genetics, Vol. 28, pp. 387—395.
15. LAMM, R. 1936. Cytological studies on inbred rye. — Hereditas XXII, pp. 217—240.
16. LESLEY, J. W. and LESLEY, M. M. 1929. Chromosome fragmentation and mutation in tomato. — Genetics, Vol. 14, pp. 321—336.
17. LEVAN, A. 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas XXVIII, pp. 177—211.
18. LEWITSKY, G. A. 1931. The morphology of chromosomes. — Bull. Appl. Bot., Genet. a. Plant-Br., Vol. 27, pp. 19—174.
19. LEWITSKY, G. A., MELNIKOV, A. N. and TITOVA, N. N. 1932. The cytology of the offspring of the 16-chromosome rye. — Bull. Lab. Genet., Vol. 9, pp. 89—96.
20. LONGLEY, A. E. 1927. Supernumerary chromosomes in *Zea Mays*. — Journ. Agric. Res., Vol. 35, pp. 769—784.
21. MCCLINTOCK, B. 1933. The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea Mays*. — Zeitschr. f. Zellforsch., Bd. 19, S. 191—237.
22. MÜNTZING, A. and PRAKKEN, R. 1941. Chromosomal aberrations in rye populations. — Hereditas XXVII, pp. 273—308.

23. POPOFF, A. 1939. Untersuchungen über den Formenreichtum und die Schartigkeit des Roggens. — Angewandte Botanik, Bd. 21, H. 4, S. 325—356.
24. RANCKEN, G. 1934. Zytologische Untersuchungen an einigen wirtschaftlich wertvollen Wiesengräsern. — Acta Agr. Fenn. 29, pp. 1—92.
25. RANDOLPH, L. F. 1928 a. Chromosome numbers in *Zea Mays* L. — Mem. Cornell Agric. Exp. Sta. No. 117.
26. — 1928 b. Types of supernumerary chromosomes in maize. — Anat. Rec. 41, p. 102.
27. TAKAGI, F. 1935. Karyogenetical studies on rye. I. A trisomic plant. — Cytologia, Vol. 6, pp. 496—501.

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EXAGGERATION OF BAR IN DROSOPHILA MELANOGASTER

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I. INTRODUCTION.

IT is well known that the effect of the sex-linked, semidominant gene Bar (*B*, located at 57,0) in *Drosophila melanogaster* is a narrowing of the eyes, and that this effect is dependent, not only on the environment (temperature), but also on modifying genes (for review of literature cf. STEINBERG, 1941 a, b). In the present article a dominant sex-linked lethal modifier will be described, which, as it makes the Bar eye still narrower, acts as an intensifier. As its symbol the letter *i* was first chosen and it has been shortly described in »Drosophila Information Service» by this letter. But the letter *i* is also found in the symbol of infrabar, and therefore the name was changed to »*exaggeration of Bar*» with the symbol *Eb*.

For aid and advice in micro-photographing we were much indebted to Dr. T. CASPERSSON when making photographs in ultra-violet light and to Dr. T. WICKBOM when photographing in ordinary light. We express here our sincere thanks.

Apart from the gene *Eb* itself the following sex-linked genes are mentioned in this article: yellow, body yellow (also classifiable among larvæ), *y*, 0,0; Hairy wing, extra bristles and hairs, *Hw*, 0,0; scute, scutellar bristles fewer, *sc*, 0,0; crossveinless, crossveins absent, *cv*, 13,7; vermilion, eyes bright vermilion, *v*, 33,0; miniature, wings very small, *m*, 36,1; garnet, eye-colour garnet pink, *g*, 44,4; forked, bristles and hairs gnarled, *f*, 56,7; Bar, eye narrow, *B*, 57,0; fused, venation confluent, *fu*, 59,5; carnation, eye-colour dark ruby, *car*, 62,5. Other characters mentioned are the deficiency Notch, with the somatic effect of notching wing tips, *N*; and the long inversion delta 49, *dl-49*. Bands in the salivary chromosomes are given numbers according to BRIDGES's map (BRIDGES, 1938).

II. ORIGIN OF Eb.

The origin of *Eb* is somewhat obscure. A female with attached *X*'s was crossed to a Bar male. Among her female offspring there were

two *XX*-detachments, which consequently were phenotypically heterozygous Bar. One of these gave, when mated to a Bar male, a quite normal offspring: 78 heterozygous and 105 homozygous *B* daughters, 60 wild type and 89 *B* sons. One of the heterozygous *B* daughters was again mated to a wild type male and gave the offspring shown in

TABLE 1. *Offspring of a heterozygous Bar female when mated to a wild type male.*

Culture number	females			males	
	+	*B*/+	*B*/*B*	+	*B*
12663	6	77	2	66	—

Table 1. It is obvious that the heterozygous *B* female must have had her *X*'s attached. The 6 wild type daughters may have been detach-

TABLE 2. *Offspring of homozygous Bar females when mated to wild type brothers. Totals from a number of inbred generations.*

females			males	
+	*B*/+	*B*/*B*	+	*B*
95[1]	1685	35936	38210	1189

ments, and the two daughters labelled as homozygous *B* females may have arisen after crossing over between *B* and the centromere. One of

TABLE 3. *Excerpt from Table 2 for a single female.*

Culture number	females			males	
	+	*B*/+	*B*/*B*	+	*B*
15558	4	2	47	91	1

these two females was crossed to wild type brothers, and the same was repeated for a number of subsequent generations (Table 2). The high percentage of detachments may be noted as well as its much higher value among females, 4,47 %, than among males, 3,02 %; and also the much greater number of sons than of daughters. Especially interesting are the wild type females. In one case there were found not less than 4 such females from a single mother (female 15558), shown separately

[1] Among these were 4 gynandromorphs.

in Table 3. Of the 95 wild type females, 25 — of which one was daughter to female 15558 — were mated to Bar males (Table 4. The males, except those from culture 15586, were only counted, not classified, but it is known that the great majority were Bar). The difference is striking: in the 24 cultures that are not from 15558 the sex-ratio was

TABLE 4. *Offspring of wild type females from Table 2 (and 3), when mated to Bar males.*

Culture number	females			males	
	+	*B*/+	*B*/*B*	+	*B*
Not from culture 15558	1275	37	—	1303	
15586 from culture 15558	—	74	34	50	—

normal, the wild type mothers had attached *X*'s and there were no daughters classified as *B*/*B*. In culture 15586 (from 15558), on the other hand, the sex-ratio was the sex-linked lethal one, the mother had not attached *X*'s, and there were a great number of daughters labelled as *B*/*B*. As the mother was wild type it seemed necessary to conclude that these »*B*/*B*»-daughters were not homozygous Bar, but that the sex-linked lethal had the dominant effect of making heterozygous Bar females look like homozygous Bar. This is the gene *Eb*, which thus had, so to say, substituted a *B* gene in a homozygous *B* female.

All wild type females, which, when crossed to Bar males, have produced daughters looking like homozygous Bar females, are descendants in direct line from female 15558, though not necessarily from female 15586. It seems thus probable that the gene *Eb* occurred as a mutation in female 15558. The most probable explanation of the 91 wild type females, Table 2 (i. e. the 95 from which the 4 of Table 3 have been substracted) is that their wild type fathers had a piece of a *Y* attached to their *X*, and that after *XY* crossing over two attached *X*'s have resulted.

III. LOCALIZATION OF Eb.

Females of the constitution *f B fu*/*Eb* were outcrossed to wild type males. The offspring are shown in Table 5. In this table the wild type males must have been exceptionals, and thus their mothers of the *XXY* constitution. The single *f fu* male may have been a contamination,

though this is very unlikely, as there were no *f fu* males used in these cultures. More probably it was a true reverse mutation from Bar to wild type.

From Table 5 it is seen that *Eb* is situated to the right of *B* and

TABLE 5. *Offspring of females of the constitution f B fu/Eb, when mated to + males.*

females	males						Total males except +
	f B fu	*f B*	*B fu*	*fu*	*f fu*	+	
31510	10941	138	13	14	1	80	11107

very close to it. When calculating the percentage of crossing over this must be made on the basis of regular males only. As the total of such males is 11107, it will be seen, according to Table 5, that the distance from *B* to *Eb* is 0,13 units and from *Eb* to *fu* 1,24 units. The distance from *f* to *B* is here 0,12 units. Thus compared with standard values the whole section from *f* to *fu*, or at least the section from *B* to *fu*, has shortened in the presence of *Eb*.

IV. THE CROSSING OVER REDUCING EFFECT OF Eb.

The shortening of the distance from *f* to *fu* in the presence of *Eb* cannot, however, be proved by a comparison with standard map values only. Therefore a special experiment was designed. This consisted in a contemporary crossing to *f fu* males of females of the following four types: *f B fu/+*, *f B fu/Eb*, *f B/fu*, *f B Eb/fu* (in the last two cases the *fu* chromosome contained also vermilion, but no classifications with respect to this gene were made). Tables 6 and 7 show the result.

It was found that a great number of non-disjunctional exceptions occurred, indicating that some mothers were *XXY*. From Table 6 it is seen that when *Eb* was not present 5 exceptional females and 193 exceptional males occurred, and when *Eb* was present there were 1 exceptional female and 19 exceptional males. The cause of the very great surplus of exceptional males is not known. When computing crossing over percentages these exceptions must not be counted. They do not therefore enter in the totals of the tables. In Table 7 it cannot be stated directly which of the flies are exceptions, as the same phenotype may have occurred through crossing over. The number of exceptions is therefore estimated in the following way. When the mother

TABLE 6. *Offspring from the crosses f B fu/+ × f fu and f B fu/Eb × f fu. (The non-disjunctional exceptions are not included in totals.)*

(The symbols in the head of the table refer as usual to the phenotypic classification. But as there are several Bar phenotypes, the symbols also take account of the genotypic constitution with regard to *B* and *Eb*. Thus, for instance, *f B/+ fu* is the symbol of females phenotypically showing the characters forked, fused, and ordinary heterozygous Bar eyes, and *f B Eb/+* is the symbol of females phenotypically showing the characters forked and eyes which correspond to the genotype *B Eb/B⁺ Eb⁺*).

Genotype of mother	females										males							
	f B/+fu	+	*f*	*B/+fu*	*f B Eb*	*f B/+*	*fu*	*B/+*	*B/Eb*	Total	*f B fu*	+	*f*	*B fu*	*f B*	*fu*	*f fu*	Total
f B fu/+	2548	2890	7	8	—	76	93	5	—	5622	2570	2850	12	9	81	80	193	5602
f B fu/Eb	2405	2695	1	7	1	43	53	—	1	5205	2442	—	—	1	42	3	19	2488

TABLE 7. *Offspring from crosses f B/v fu × f fu and f B Eb/v fu × f fu.*

(Not classified with respect to *v*. The non-disjunctional exceptions are not included in totals. Cf. text about the estimation of the numbers of exceptions. Cf. also Table 6 about the symbols in the head of the table).

Genotype of mother	females										males						
	f B/+	*f B Eb/+*	*fu*	*f fu*	*B/+*	*B Eb*	*f B/+fu*	*f B Eb fu*	+	Total	*f B*	*fu*	*f fu*	*B*	*f B fu*	+	Total
f B/v fu	3627	—	3430	5	8	—	84	—	87	7238	3401	3554	19	5	67	91	7123
f B Eb/v fu	—	3181	3112	6	—	7	6	40	79	6430	—	3207	15	—	1	54	3268

is a *f B/fu* female, the phenotypically *B/+* daughters may be crossovers between *f* and *B* or exceptions. As there are 5 daughters in the crossover class *f fu*, the same number of crossovers is supposed among the *B/+* daughters, leaving 3 as exceptions. For the same reason, among daughters of *f B Eb/fu* mothers, only 1 *B Eb/+* is reckoned as an exception. The same method is used when estimating male exceptions, only that, when *Eb* is present in the mother, there is only one male crossover class in each interval. Owing to the fact that there are 6 *f fu* daughters, 9 of the 15 *f fu* males are reckoned as exceptions. Obviously there may be errors in these estimates. But such errors can affect crossing over percentages between *f* and *B* only, not between *B* and *fu*.

Numbers of crossovers and crossingover percentages are found in Table 8, and in Table 9 χ^2 tests of significance are made.

For the interval *f*—*B*, the χ^2 values correspond to P values all above the 5 per cent point. The male χ^2 value, 2,87, seems nevertheless rather high to be due to chance alone, but may be a result of errors in estimates of numbers of exceptions. As, further, there is no appreciable difference between the two sexes, χ^2 being 0,87, there seems to be no reason to suppose any influence from *Eb* on crossing over between *f* and *B*. This could also, on a priori grounds, be foreseen, as *Eb* has been located to the right of *B*.

In the case of the interval *B* to *fu*,

TABLE 8. *Crossing over percentages with and without Eb.*

	females					males					Total				
	Cross overs				Total	Cross overs				Total	Cross overs				Total
	f—*B*		*B*—*fu*			*f*—*B*		*B*—*fu*			*f*—*B*		*B*—*fu*		
	numbers	%	numbers	%		numbers	%	numbers	%		numbers	%	numbers	%	
Eb-not present	25	0,19	340	2,64	12860	31	0,24	319	2,51	12725	56	0,22	659	2,58	25585
Eb-present	20	0,17	222	1,91	11635	7	0,12	100	1,74	5756	27	0,16	322	1,85	17391

on the contrary, the crossing over reducing effect of *Eb* is very clearly demonstrated by the high values of χ^2. No difference between the two sexes is at hand.

The nature of the »gene» *Eb* will be discussed later, but the fact

TABLE 9. *χ^2 values for differences in crossing over percentages when Eb is present and not present.*

	f—B	*B—fu*	Degrees of freedom
females	0,17	14,75	1
males	2,87	10,59	1
Total	3,04	25,34	2
χ^2 for females and males	2,17	24,34	1
Difference	0,87	1,00	1

that it reduces the per cent of crossing over suggests that it occupies a certain length within the chromosome. In Table 10 some of the figures of Tables 6 and 7 are put together. According to them the left limit of *Eb* lies 0,07 units to the right of Bar, and its right limit 1,67 units

TABLE 10. *Crossing over percentages among males, when mothers are heterozygous with respect to Eb. Data from Tables 6 and 7.*

Cross overs				Total males
B—Eb		*Eb—fu*		
numbers	%	numbers	%	
4	0,07	96	1,67	5756

to the left of fused. The corresponding values found from Table 5 (compare Section III) were 0,13 and 1,24 units. As Table 8 shows, there was found 2,51 per cent male crossovers between *B* and *fu* when *Eb* was not present (standard value of map distance from *B* to *fu* 2,5 units). The total length of the »gene» *Eb* should thus, according to the special experiments (Tables 6—10), be $2{,}51 - 0{,}07 - 1{,}67 = 0{,}77$ map units. According to the results of Table 5 the length should be $2{,}5 - 0{,}13 - - 1{,}24 = 1{,}13$ units. The genetic length of *Eb* may thus finally be taken as being about 1 map unit.

V. CYTOLOGY OF Eb.

A rather great number of slides of salivary gland chromosomes have been made. Micro-photographs have also been made with normal as well as with ultra-violet light. The female larvæ have had *Eb* in different combinations of *B*, but we have got the clearest pictures from *Eb*/+ females when microscoped in ordinary light. When making slides of these larvæ we have used *Eb*/*dl-49*, *y Hw* m^2 g^4 females, which have been mated to *dl-49*, *y Hw* m^2 g^4 males. Thanks to the inversion *dl-49*, in which the breaks are at 4 D 7—4 E 1 and 11 F 2—F 4, all or practically all non-yellow female larvæ have the desired constitution, viz. (when omitting the genes which from the standpoint of *Eb* are irrelevant) the constitution *Eb*/+.

Unfortunately, we are not able to draw any conclusive inference as to the cytological nature of *Eb*. In fact, in the majority of the slides we do not find any certain difference in the Bar region between *Eb*/+ larvæ and normal wild type female larvæ (Fig. 1 *a*, *b*). In a few cases, however, the pictures seem to indicate a very short deficiency, comprising one, or possibly two, of the faint bands 16 A 5 and 16 A 6 (Fig. 1 *c*, *d*). But it is not impossible that in these cases the similarity of a deficiency is due to a slight contortion of the two chromosomes. Just as we cannot find any reliable difference between the chromosomes of +/+ and *Eb*/+ females, we are not able from our slides to ascertain any difference between the chromosomes of *B*/+, *B*/*Eb* and *B Eb*/+ females. Furthermore, the two chromosomes of the *Eb*/+ larvæ are in the great majority of cases well synapsed in this region. This must mean that *Eb* is not an inversion. Very short inversion heterozygotes are not expected to form inversion loops. And if, for instance, only two faint bands are inverted, it is hardly thinkable to distinguish the two chromosomes from each other. But it must be presumed that in all such heterozygotes the chromosomes remain unsynapsed within the short inversion region.

The conclusions that we can draw from our studies of the cytology of *Eb* are thus so far of a negative kind: if the *Eb*-containing chromosome is at all different from a wild type *X* chromosome, this difference must be very inconspicuous. *Eb* is certainly not a large deficiency, inversion, or duplication. As there furthermore is no synapsis either between different parts of the *X* chromosome or between the *X* and an autosome, *Eb* is not involved in any transposition or translocation.

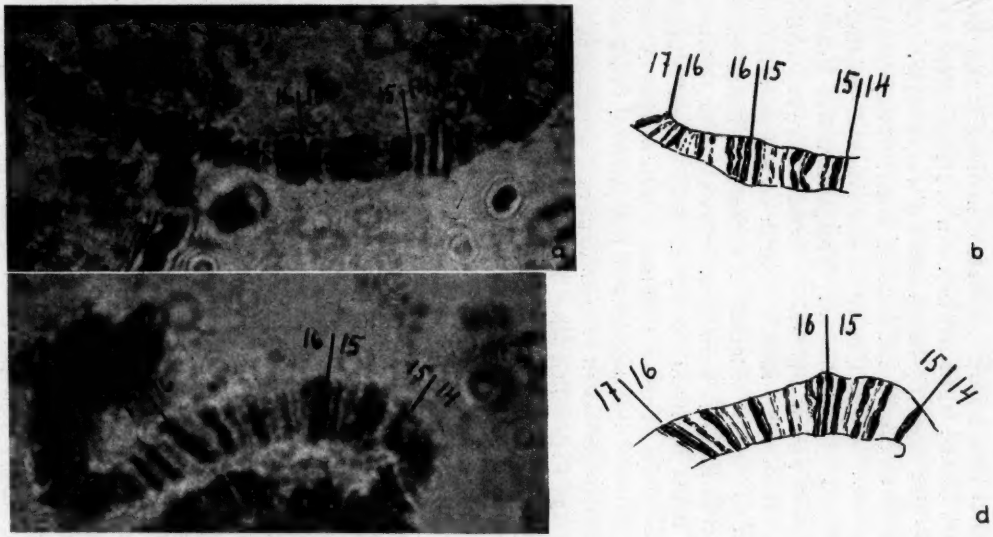

Fig. 1. Salivary chromosomes of *Eb*/+ females. — *a*, microphotograph from a cell which does not show any certain difference from wild type. — *b*, drawing from the same cell as *a*. — *c*, microphotograph from a cell which indicates a deficiency. — *d*, drawing from the same cell as *c*. The drawings were made directly from the microscope when focusing section 16 at different levels.

VI. POSITION EFFECT OF Eb. FACET COUNTS.

The experiments which have been described above started from females having *B* in one *X* and *Eb* in the other. When observing the first crossover and thus getting a female containing *B* and *Eb* in the same *X*, her eyes looked still narrower. In order to ensure a reliable study of this possible case of position effect it was, however, necessary first to produce standardized, i. e. isogenic, lines. These were obtained in the following way. A wild type strain was inbred by brother—sister pairing for at least 11 generations. This strain was called: series hom. +. Thereafter yellow attached *X*'s females were repeatedly back-crossed to males from series hom. + and in such a way that for each new generation of yellow attached *X*'s females, males of a later generation of series hom. + were used. This series of yellow females crossed to wild males constituted series A. After at least 5 generations females of series A were crossed to males, which in their *X* contained *sc cv v car* and different combinations of *f*, *B*, and *fu*. Male offspring were for each generation mated to females of new generations of series A. These crossings were called series B. In cases when *Eb* had to be introduced into isogenic lines additional crosses had to be made. For instance, to produce *f B Eb* standardized chromosomes, the following crosses were made:

1. *f B Eb*/+ × *sc cv v f car*
2. *f B Eb*/*sc cv v f car* × *sc cv v f car*
3. *sc cv v f B Eb*/*sc cv v f car* × *sc cv v f car*
4. *sc cv v f B Eb car*/*sc cv v f car* × *sc cv v f car*

The males used thereafter were from series B. After at least 12 generations of series B had been passed some crosses were made between some of the lines thus standardized, whereupon the earliest facet counts began. For most of the facet counts still more inbreeding generations preceded the counts. All cultures have been bred in incubators at 25° C, with a maximum range of variation of ± 1° C. In all standardized cultures the genes *sc*, *cv*, *v*, and *car* were present. They are not explicitly mentioned in the following descriptions.

The technique of counting facets has been that used by earlier workers, though with some slight modifications. Etherized flies were, for a shorter or longer time, put in 95 % alcohol. The corneas were dissected in water, cleaned, and transferred to a 5 % KOH solution. (The KOH is not quite necessary and may be omitted.) 4 to 8 corneas were placed on each slide in aceto-carmine, and covered by a cover-slip.

Other stains may of course be used, but the aceto-carmine fills out the facets and gives good results. With the aid of a Bausch and Lomb micro-projector an image of 500 times magnification was obtained. In this micro-projector the slides were placed in a vertical position, but owing to the stickiness of the aceto-carmine the corneas rested where they were mounted. The image was projected on a paper, each facet-image was marked by a dot, and these dots were thereupon counted. If desired, the paper can be sensitized to enable a photograph to be obtained. When counting facets of the broader eyes fine incisions have to be made in order to make the eyes less spherical. Only facets from female eyes have been counted, and only from one eye of each female.

The first eyes to be counted were from *B Eb*/+ females. It was, however, later decided to make counts on *B Eb*/+ and *B*/*Eb* eyes from females hatched in cultures which had been incubated simultaneously. Therefore, counts of *B Eb*/+ were made on two different occasions. The difference between the two means, 41,01 and 40,02, is very small, and a statistical analysis showed that the mean square within counts was (insignificantly) greater than that between counts. In the following, therefore, these two counts are pooled. The facet counts are summarized in Table 11.

TABLE 11. *Facets counts.*

(All females were taken from standardized lines and were thus homozygous with respect to *sc cv v car*. In addition, all except the recovered *B*/*Eb* were homozygous with respect to *f*. The recovered *B*/*Eb* did not carry *f*.)

Genotype of females	Number of eyes counted	Mean facet number	χ^2 test of normality of grouped distribution	
			Number of facets per grouping unit	P
+/+	29	$667{,}45 \pm 8{,}90$		
Eb/+	26	$723{,}88 \pm 5{,}56$		
B/+	37	$377{,}78 \pm 11{,}16$		
B/*Eb*, original	107	$84{,}27 \pm 1{,}76$	5	0,2
B Eb/+	190	$40{,}57 \pm 0{,}47$	3	0,2—0,3
B/*Eb* recovered 1	102	$90{,}18 \pm 1{,}44$		
B/*Eb* recovered 2	116	$89{,}79 \pm 1{,}29$		
B/*Eb* recovered 1 and 2 pooled	218	$89{,}97 + 0{,}96$	5	0,3—0,5
B/*B*	62	$80{,}68 \pm 1{,}61$	5	0,2—0,3
B Eb/*B*	51	$28{,}51 \pm 0{,}57$	2	0,2—0,3

The principal result is that the position effect is clearly demonstrated. From Table 11 it is in fact to be seen that *B*/*Eb* females (*B*/*Eb*, original) have 84,27 ± 1,76 facets, whereas *B Eb*/+ females have only 40,57 ± 0,47 facets.

It was decided to study whether *Eb* had any effect when not cooperating with *B*, it being conceivable that *Eb*/+ eyes have somewhat fewer facets than +/+ eyes, though without this being detectable when the flies are inspected in ordinary classifying works. The surprising result (see Table 11) was, however, the reverse, viz. +/+ eyes had fewer facets than *Eb*/+ eyes. As the ratio of the mean square between counts to the mean square within counts was found to be 27,41, the difference is highly significant. This peculiar result must, before it can be taken as granted, be checked by further counts. But, as the counts on these large eyes are the most laborious ones, and as these comparisons are of minor importance in this connection, no more such counts have been performed. It may, however, be wise to call attention to the fact that temperature is not the only environmental factor which influences the facet number. Crowding and dryness of food also seem to lower this number, as we have found females in old stock bottles of wild type with only about 500 facets. Now, in ordinary culture bottles, it is impossible to ascertain the same number of flies in every bottle. And it is thus possible that there were more flies in the bottles from which the +/+ counts were made than in those from which the *Eb*/+ counts were made. It seems nevertheless difficult to explain the big difference between the two means, 56,43 facets, as being due solely to this environmental cause. And there is not the slightest reason to believe that the more than twice as great a number of facets that we have found in *B*/*Eb* flies when compared with *B Eb*/+ flies should be due to this cause.

The concept of position effect was first introduced by STURTEVANT (1925) in his study of unequal crossing over. Since then a great number of cases of position effect have been reported. Most of them are chromosomal rearrangements, that is to say, changed phenotypical effects are the result of rearrangements within the chromosomes. But the chief difficulty of getting conclusive proof that these changes in phenotypes are really due to position effects depends on the impossibility of securing rearrangements in the reversed order from the new chromosomal configuration to the original one. Take Baroid as a typical example (DOBZHANSKY, 1932). This is a translocation between the first and the second chromosomes with the breakage in the *X* chromosome within

the Bar region at 16 A 1, 2 (MORGAN, BRIDGES and SCHULTZ, 1936). The Baroid flies have eyes of the Bar type, and this changed phenotype is, on the position effect hypothesis, explained as a result of the Bar locus getting new neighbour genes. But the effect could of course also be explained by the assumption that a mutation occurred simultaneously with the translocation. To prove that the position effect hypothesis is the correct explanation, a reverse translocation from the Baroid to the normal chromosome configuration must be produced accompanied by the backward shift in phenotype from Bar type to wild type eye. But even if a reverse translocation could be produced it is probably impossible to ascertain that the original chromosome has been exactly restored. In one case a much more conclusive proof has been given. DUBININ and SIDOROV (1935) described a translocation between the fourth and the third chromosomes with the point of breakage in the third chromosome just to the right of the locus of the mutant hairy. Flies with one unbroken third chromosome containing hairy and one broken chromosome carrying its wild allele are phenotypically hairy. The point of breakage is, however, situated far enough from hairy to allow crossing over between the locus of hairy and the breakage. DUBININ and SIDOROV were thus able to secure flies in which the unbroken chromosome contained the wild allele, and the broken one carried the gene hairy. Such flies were wild type. From these flies again the first condition could once more be restored through crossing over, and the result was hairy flies. A case similar in every way to that of hairy has been reported for the third chromosome wing character curled by PANSHIN (cited from the textbook by STURTEVANT and BEADLE, 1940, p. 225).

In the offspring of the original female combination *B/Eb* there occurred through crossing over a small amount of *B Eb/+* females (Table 6). The possibility of recovering the original *Eb* chromosome through crossing over in these *B Eb/+* females was thus expected. In order to achieve this *f B Eb/f* females were mated to *fu* males. (All flies here and in the following crossings were as before from the standardized lines, thus also containing *sc cv v car.*) *f B Eb/fu* daughters were crossed to *f fu* males and all daughters of this mating, which were phenotypically wild type, were crossed individually to *B* males. Such wild type daughters are of two distinct kinds, a larger part +/+ and a smaller part *Eb/+*, and only after crossing them to the *B* males is it possible to make a distinction between them. +/+ females must, when crossed to *B* males, give female offspring all having eyes of the hetero-

zygous Bar type. *Eb*/+ females, on the contrary, will produce daughters, half of which have heterozygous *B* eyes and the other half the narrow *B*/*Eb* eyes. Furthermore, the former females give the normal sex-ratio and the latter the sex-linked lethal ratio. Thus, if in any culture bottle about half of the daughters have the narrow *B*/*Eb* eyes, an *Eb* chromosome has, through crossing over, been recovered from the *B Eb* chromosome. This happened with two different bottles, giving two recovered strains. In order, however, to get a larger number of flies before the facet counts were undertaken, the *B*/*Eb* females from the two strains were crossed to wild type males, after which the phenotypically wild type daughters were again crossed to *B* males, and facet counts made on their *B*/*Eb* daughters (Table 11, *B*/*Eb* recovered 1 and 2). These two recovered *B*/*Eb* strains gave the facet numbers $90{,}18 \pm 1{,}44$ and $89{,}79 \pm 1{,}29$. Obviously there is no statistical difference between these two figures (in fact, the mean square within strains was greater, but of course insignificantly so, than the mean square between strain means). The figures from the two strains may therefore be pooled, giving a pooled mean of $89{,}97 \pm 0{,}96$ facets.

We thus see that a recovered *Eb* chromosome has, in co-operation with a *B* chromosome, an effect similar in kind to that of the original *Eb* chromosome. But though the effect is similar it is not identical, since the mean facet number in the recovered *B*/*Eb*, being 89,97, is larger than the mean facet number, 84,27, of the original *B*/*Eb*. In this case the ratio of the mean squares between means and within original and recovered strains is 9,62, which, as the degrees of freedom are 1 and 323, corresponds to a significance level, P, of about 0,002. The difference in facet number between the original and the recovered *B*/*Eb* strains must accordingly be judged as well significant.

In this connection it may, however, be wise to reconsider under what conditions such statistical tests are supposed to be reliable. It is known that in many cases the means of samples are distributed normally even when these samples are drawn from non-normal populations. But the populations must not be too far from normality. When, therefore, as in our comparison between the original and the recovered *B*/*Eb*, we find that the difference between the two means corresponds to a calculated value of P not less than 0,002 this has a meaning only if the means are distributed normally. In order to test this, all facet counts with more than 50 eyes per sample were fitted to the normal distribution. This was done after grouping, using 2, 3 or 5 facets as grouping unit, 2 for the smaller and 5 for the larger mean facet

numbers. The comparison between the observed number of facets in each group with the expected ones was made by the aid of the χ^2 test. When doing so we have to remember that the expected frequencies have been calculated from: the observed mean, the standard deviation (by calculating the limits of the grouping intervals of the fitted distribution), and the total number of facets observed. Thus if the number of groups is *n*, the degrees of freedom are *n*—3. A small error has been introduced by the fact that there are often found some eyes, though very few in number, with exceptionally high facet numbers. These eyes then belong to different groups, and therefore these groups at the end of the right tail have been pooled prior to fitting. In order that the expected frequencies in no group shall be less than 5 (FISHER, 1936), some of the tail-end groups have also been pooled after fitting. The result of the tests are shown in Table 11, and we do not seem to have any reason to be suspicious of the significativeness of the difference between the facet means of the original and recovered *B/Eb*:s. It is impossible to settle with certainty the reason for this difference. But there are several causes which may have been effective.

The cultures from which the counts in the two recovered strains were made were raised contemporarily but about two months after the raising of the cultures from which the counts in the original strain were made. The culture conditions and the temperature in the incubator were, as far as possible, identical on the two occasions, and it does not seem probable that such kinds of environment caused the difference. It does not seem probable either that differences in crowding were the reason, since on both occasions many parallel cultures were raised and flies were taken from various of them. It is more likely that the isogeneity was not perfect enough to ensure complete identity with respect to modifying genes. The desired isogeneity was, as described above, built up by introducing the genes *sc*, *cv*, *v*, and *car* into the *X* of the standardized lines. But in the neighbouring region of the locus of *Eb* itself, it was, for obvious reasons, necessary to work with standardized strains which differed within this region, and so »isogenic» lines with different combinations of *B*, *Eb*, *f*, and *fu* had to be synthesised. Now, the original *B/Eb* strain was homozygous with respect to *f*, whereas the recovered strains were homozygous with respect to its normal allele. The consequence of this is that there existed a small chromosomal difference between the original and the recovered strains, and it is conceivable that this also involves a difference with respect to modifying genes. Furthermore, it must be remembered that the mode of synthesis-

ing the standardized strains is by no means absolutely reliable. There may be minor differences between the different strains. This is also to some extent substantiated by our impression that the recovered *B/Eb* flies had a somewhat higher viability than the original *B/Eb* flies. Such a difference in viability must be due to different genetic constitution, and, even if this, strictly speaking, does not involve any differences with respect to Bar modifiers, the higher viability may give larger flies with larger eyes and thus with more facets.

Finally, the two crossing-over processes which led from the original *B/Eb* to the recovered ones may have brought about the difference in question. The reflections which may be made concerning this possibility belong, however, more correctly to the discussion in the next section of this article, and the problem will therefore be dealt with there.

Concluding the above considerations, we may state that through crossing over in a *B/Eb* female, followed by a crossing over in a reverse direction, an *Eb* chromosome may be restored, with, if not identical, then at least with very similar properties as those of the original *Eb* chromosome. The list of the reliable cases of position effects has thus, as it seems, got a new and very clear member.

VII. DISCUSSION.

The fact that *Eb* reduces the per cent of crossing over between *B* and *fu* suggests a deficiency or an inversion. The possibility of inversion may, however, be kept out of discussion for reasons given in Section V. With reference to the hypothesis of a deficiency, compare *Eb*, for instance, with the N8-deficiency (MOHR, 1923). This deficiency has a genetic length, i. e. a crossing over reducing effect, of 3,8 map units, thus about four times as many units as *Eb*. If there is some closer correspondence between map length and salivary chromosome length (which of course is not necessary; MATHER, 1936), *Eb*, if a deficiency, should occupy about a quarter of the number of bands which are included in N8. The breaks of N8 are at 3 C 1, 2 and 3 D 5, 6 (SLIZYNSKA, 1938) and thus include about 18 bands. Under the above assumption, therefore, *Eb* should occupy 4 to 5 bands, which it certainly does not.

It is known that Bar is a duplication of the 5 or 6 first bands in section 16 A (BRIDGES, 1936; MULLER, PROKOFJEVA-BELGOVSKAJA and KOSSIKOV, 1936). As stated in the description of the origin of *Eb*, it must have occurred in a *B/B* female. Now, *Eb* does not have the Bar section repeated twice, and at the mutation from Bar to *Eb*, therefore,

one of the sections must have disappeared. It is obvious that this can happen through an unequal crossing over (STURTEVANT, 1925). If this unequal crossing over was due, not only to an oblique pairing of the chromosomes, i. e. a synapsis between the left segment in one Bar gene with the right segment in the other Bar gene, but to a really unequal or illegitimate crossing over (such as must have happened at the original mutation from wild to Bar), then it is quite conceivable that a short deficiency could have occurred at the same time. However that may be, it is important to remember that when *Eb* originally occurred in a *B*/*B* female one Bar segment was lost. Or so much of this segment must at any rate have been lost that it is extremely difficult to find any remnants of it in the salivaries — and we have in fact been unable to find any such.

But there is one other reason why a deficiency nature of *Eb* is very unlikely. The first case of a deficiency to be described in the literature, and in which the word deficiency was originally introduced, was Bar-deficiency (BRIDGES, 1917). The deficient section included here the locus of forked. According to BRIDGES, »it was found that the female carrying bar in one *X* and bar-deficiency in the other was in somatic appearance like the normal bar heterozygote and not like the homozygous bar female». At that time the salivary chromosomes were not known and consequently the duplicate nature of Bar was also unknown. But BRIDGES's statement is in fact in good accordance with this duplicate nature, if we assume that the deficient region included the distal one of the two segments. For in that case the deficient chromosome contains one of the Bar segments, just as does a wild type chromosome. If this deficiency included both segments, a female heterozygous Bar/Bar-deficiency should have two segments in one *X* and none in the other, i. e. the same number of Bar segments as have wild type females. The eyes of the Bar/Bar-deficiency females should therefore under these conditions presumably be round, or, as the result of a position effect, only slightly of the Bar type. In the »Drosophila Information Service», No. 9, the list of mutants contains 10 Bar-deficiencies, all of which have occurred after X-raying Bar or double infra-bar males. In 9 of the cases it is stated that the eyes are normal, whereas in one case (from a B^l B^l male) the eyes are »large like B^l presumably one B^l was removed». Unfortunately no information is given about the eyes of Bar/Bar-deficiency females, and as the present authors do not possess such females, we do not know which eye shape they have. We can only presume that, as in BRIDGES's deficiency and for the same reason, the

eyes are not as narrow as the eyes of *B*/*B* females. If they really had been of such a nature, this most probably would also have been mentioned in the »Drosophila Information Service».

In Table 12 we have put together some of the facet counts of STURTEVANT (1925) in order to compare them with our own counts. (In STURTEVANT's Table 23 ± the probable error has been added to the mean facet numbers. But we have as usual used the standard error. In Table 12, therefore, the probable errors from STURTEVANT's Table 23

TABLE 12. *Comparisons between facet counts from* STURTEVANT (*1925*) *and from the present article.*

Genotype of females	Figures from STURTEVANT		Figures from this article	
	Mean facet number	Number of eyes counted	Mean facet number	Number of eyes counted
+/+	$779{,}4 \pm 6{,}08$	25	$667{,}45 \pm 8{,}90$	29
B/+	$358{,}4 \pm 11{,}72$	25	$377{,}78 \pm 11{,}16$	37
B/*B*	$68{,}12 \pm 1{,}62$	50	$80{,}68 \pm 1{,}61$	62
B/*Eb* original	—	—	$84{,}27 \pm 1{,}76$	107
BB/+	$45{,}42 \pm 0{,}36$	161	—	—
B Eb/+	—	—	$40{,}57 \pm 0{,}47$	190
BB/*B*	$36{,}43 \pm 0{,}44$	51	—	—
B Eb/*B*	—	—	$28{,}51 \pm 0{,}57$	51

are divided by 0,6745.) As through the standardization our isogenic stocks must have been made homozygous with respect to in part other modifying genes than STURTEVANT's stocks, it is not to be expected that there should be found the same average facet numbers in corresponding Bar-combinations. Somewhat surprising is the fact that, whereas his +/+ females have more facets than ours, his *B*/+ and *B*/*B* females have fewer facets than our corresponding females.

More interesting to note is that the effect of *Eb*, in the presence of *B*, is of the same nature as that of *B* itself. For instance, the difference between our own counts of *B*/*B* and *B*/*Eb* (original) is without statistical significance. (The ratio of the mean square between the two genotype means and within genotypes is 1,86 with the two degrees of freedom 1 and 167. As shown above, there is a statistically significant difference between the original and recovered *B*/*Eb*, and correspondingly there is also one between our counts of *B*/*B* and the recovered *B*/*Eb*. In this case the ratio of the two mean squares is 21,70 with the degrees of freedom 1 and 278.) Further, comparing STURTEVANT's *BB*/+ with

our *B Eb*/+ or his *BB*/*B* with our *B Eb*/*B*, it is found that, though there are differences which are statistically significant, the compared genotypes nevertheless have facet numbers which are of the same order of magnitude.

For the moment we have no more comparisons available between our own standardized stocks, but we hope to get such later. The comparisons already made suggest, however, rather strongly that the »gene» *Eb*, in some way or other, is allelic to the »gene» *B*. In our cytological studies we found some indications of *Eb* being a deficiency within the Bar segment, though reasons of a more general genetic kind did contradict this view. However that may be, we may well suppose that *Eb* is situated within the Bar segment. Such a supposition may at first sight seem incompatible with the fact that *Eb* has been localized to the right of *B*. But if *Eb* lies within the Bar segment so close to its right border that there hitherto has not occurred any crossings over between *Eb* and this right border, the result will just be such a localization to the right of *B*. When arguing in this way we have tacitly supposed that as soon as there is a duplication of the Bar segment, the Bar phenotype results. In this way *Eb* could simply be said to be an ordinary modifier of *B* situated within a Bar segment and, in the presence of two such segments, showing a position effect of the known Bar type. (In this case *Eb* should perhaps be looked upon as a recessive.) It may also be of some interest to note that the per cent of crossing over between *B* and *Eb*, which according to Table 5 was 0,13 and according to Table 10 was 0,07, is of the same order of magnitude as the per cent of unequal crossing over found by STURTEVANT (1925).

If there can be found one modifier of *B* within a Bar segment there may of course be found several modifiers. And so we come back to the possibility of the difference between the original and recovered *B*/*Eb* being due to the crossings over between *B* and *Eb*. If such modifiers do exist, it is not to be supposed that our »isogenic» stocks are really homozygous and isogenic with respect to them. But heterozygosity means possibility of recombining different alleles through crossing over. And as both recovered *B*/*Eb* strains were received through crossing over within one and the same *B Eb*/+ strain, it is quite conceivable that the same combination occurred in both recovered *Eb* chromosomes, giving the two recovered strains of *B*/*Eb* the same facet number though different from the number in the original strain.

Through our last considerations we have obviously from a new point touched such problems as that of step allelomorphism (SERE-

BROVSKY, 1930) and the divisibility of the genes (RAFFEL and MULLER, 1940). But to go further into the matter at the present stage would probably mean no more than resorting to mere conjectures. We hope, however, soon to be able to present more facts about *Eb*, which perhaps will give us a better opportunity for discussing these interesting problems.

SUMMARY.

1. A sex-linked lethal gene *Eb* is described, which, in co-operation with Bar, makes the eyes still narrower.

2. *Eb* has a position effect of the same kind as that of *B*, *B Eb*/+ females having narrower eyes than *B*/*Eb* females. In the presence of *B*, *Eb* has also an effect very similar to that of *B* itself. Thus the facet numbers of *B*/*B*, *BB*/+ and *BB*/*B* females are of the same order of magnitude as those of *B*/*Eb*, *B Eb*/+, and *B Eb*/*B* respectively.

3. Through crossing over experiments *Eb* is localized just to the right of *B* and, in the presence of *B*, *Eb* reduces the crossing over per cent between *B* and *fu* by about one unit.

4. *Eb* occurred in a homozygous Bar female. Cytological studies show that *Eb* does not have the Bar segment duplicated. Some slides indicate that *Eb* is a deficiency within a Bar segment, but there are also reasons against such a supposition.

5. The nature of *Eb* as being a modifier of Bar situated within a Bar segment is discussed.

LITERATURE CITED.

1. BRIDGES, C. B. 1917. Deficiency. — Genetics 2: 445—465.
2. — 1936. The Bar »gene» a duplication. — Science 83: 210—211.
3. — 1938. A revised map of the salivary gland *X*-chromosome. — Journ. of Hered. 29: 11—13.
4. DOBZHANSKY, T. 1932. The baroid mutation in *Drosophila melanogaster*. — Genetics 17: 369—392.
5. DUBININ, N. P. and SIDOROV, B. N. 1935. The position effect of the hairy gene. — Journ. of Biol. 4: 555—568.
6. FISHER, R. A. 1936. Statistical methods for research workers. Sixth edition. — Oliver and Boyd, Edinburgh.
7. MATHER, K. 1936. The determination of position in crossing-over. I. *Drosophila melanogaster*. — Journ. of Genet. 33: 207—235.
8. MOHR, O. L. 1923. A genetic and cytological analysis of a section deficiency involving four units of the *X*-chromosome in *Drosophila melanogaster*. — Zeitschr f. ind. Abst.- u. Vererbl. 32: 108—232.

9. MORGAN, T. H., BRIDGES, C. B. and SCHULTZ, J. 1936. Report of investigations on the constitution of the germinal material in relation to heredity. — Carn. Inst. Wash. Year Book 35: 289—297.
10. MULLER, H. J., PROKOFJEVA-BELGOVSKAJA, A. A. and KOSSIKOV, K. V. 1936. Unequal crossing-over in the Bar mutant as a result of duplication of a minute chromosome section. — C. R. Acad. Sci. U.R.S.S., N. S. 1: 87—88.
11. RAFFEL, D. and MULLER, H. J. 1940. Position effect and gene divisibility considered in connection with three strikingly similar scute mutations. — Genetics 25: 541—583.
12. SEREBROVSKY, A. S. 1930. Untersuchungen über Treppenallelomorphismus. IV. Transgenation scute-6 und ein Fall des »Nicht-Allelomorphismus» von Gliedern einer Allelomorphenreihe bei *Drosophila melanogaster*. — Wilh. Roux' Arch. f. Entw.-mech. 122: 88—104.
13. SLIZYNSKA, HELEN. 1938. Salivary chromosome analysis of the white-facet region of *Drosophila melanogaster*. — Genetics 23: 291—299.
14. STEINBERG, A. G. 1941 a. A reconsideration of the mode of development of the Bar eye of *Drosophila melanogaster*. — Genetics 26: 325—346.
15. — 1941 b. Studies on modifier of Bar. A mutant which affects the expression of the Bar mutation of *Drosophila melanogaster*. — Genetics 26: 440—451.
16. STURTEVANT, A. H. 1925. The effect of unequal crossing-over at the Bar locus in *Drosophila*. — Genetics 10: 117—147.
17. STURTEVANT, A. H. and BEADLE, G. W. 1940. An introduction to Genetics. — W. B. Saunders Company, Philadelphia and London.

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CHARACTERISTICS OF TWO HAPLOID TWINS IN DACTYLIS GLOMERATA

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As described in previous papers (MÜNTZING, 1937 a, 1938 a) plants with deviating chromosome numbers may be obtained by selection of twin seedlings in different species. The most frequent deviation is represented by triploids, these having a chromosome number 50 per cent higher than the typical number of the species. However, haploids may also be produced in this way and were, indeed, obtained in the following species: *Triticum vulgare*, *Hordeum vulgare*, *Phleum pratense*, *Dactylis glomerata*, *Poa pratensis*, and *Solanum tuberosum* (MÜNTZING, 1938 a, Table 1).

In the present report two haploids of *Dactylis glomerata* will be briefly considered. They were produced with a good deal of difficulty and were the only aberrants among 198 twin plants examined in this species. In both cases the other member of the pair was a normal *glomerata* plant, having $2n = 28$. Both haploids were controlled to have $2n = 14$ in the root tips. In the twin pair »1060», first obtained, the morphological difference between the haploid and its normal sister seedling was found to be very great. This is evident from Fig. 1, showing the tiny haploid to the right of the normal and vigorous sister twin, having $2n = 28$. These plants were cultivated in the greenhouse, and other clone plants were grown in the field. Measurements of plant height, panicle length, thickness of culm and leaf dimensions were made. As regards height and panicle length the haploid had less than half the dimensions of the diploid, and the leaves, too, were shorter and narrower. The culms and panicle branches were much thinner in the haploid and the leaf sheaths less scabrid. The spikelets, finally, contained about the same number of flowers in both twins, but in the haploid they were almost without hairs and had a very short acumen at the flower glumes. In the normal sister twin, on the contrary, the spikelets were quite hairy and had long acumens. This is evident from Fig. 2.

These morphological details are mentioned because the properties of the haploid *glomerata* plants are of much interest with regard to the

nature of the species differences between *Dactylis glomerata* and *D. Aschersoniana.* As shown in detail by MÜNTZING (1937 b) *D. glomerata* is an autotetraploid, the genomes of which are quite homologous to the genomes of the closely related diploid species *D. Aschersoniana.* It was also concluded (l. c.) that the morphological species differences between *glomerata* and *Aschersoniana* are essentially caused by the difference in chromosome number. Under such circumstances haploid (or more

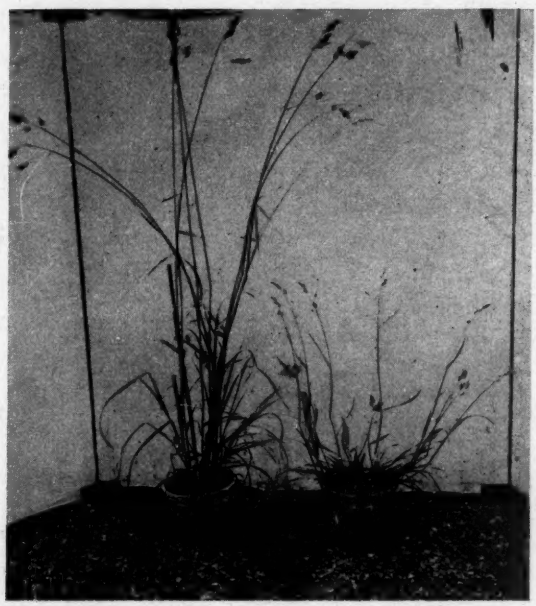

Fig. 1. The twin pair »1060» of *D. glomerata.* To the right, the haploid twin with $2n = 14$; to the left, the normal twin with $2n = 28$.

correctly: polyhaploid) plants of *D. glomerata,* having the same chromosome number ($2n = 14$) as *D. Aschersoniana,* should be expected to be morphologically rather similar to *Aschersoniana.* They should also have a normal meiosis with seven bivalents.

D. glomerata, it is true, is a gigas type in relation to *D. Aschersoniana,* but the haploid in the twin pair »1060» was much less vigorous than any type of *D. Aschersoniana.* In some morphological respects, however, there was a rather interesting similarity between the haploid twin and *D. Aschersoniana.* This is especially true of the spikeletes,

which were almost devoid of hairs and had short acumens just as in *Aschersoniana* (Fig. 2). Thus, though the haploid *glomerata* under

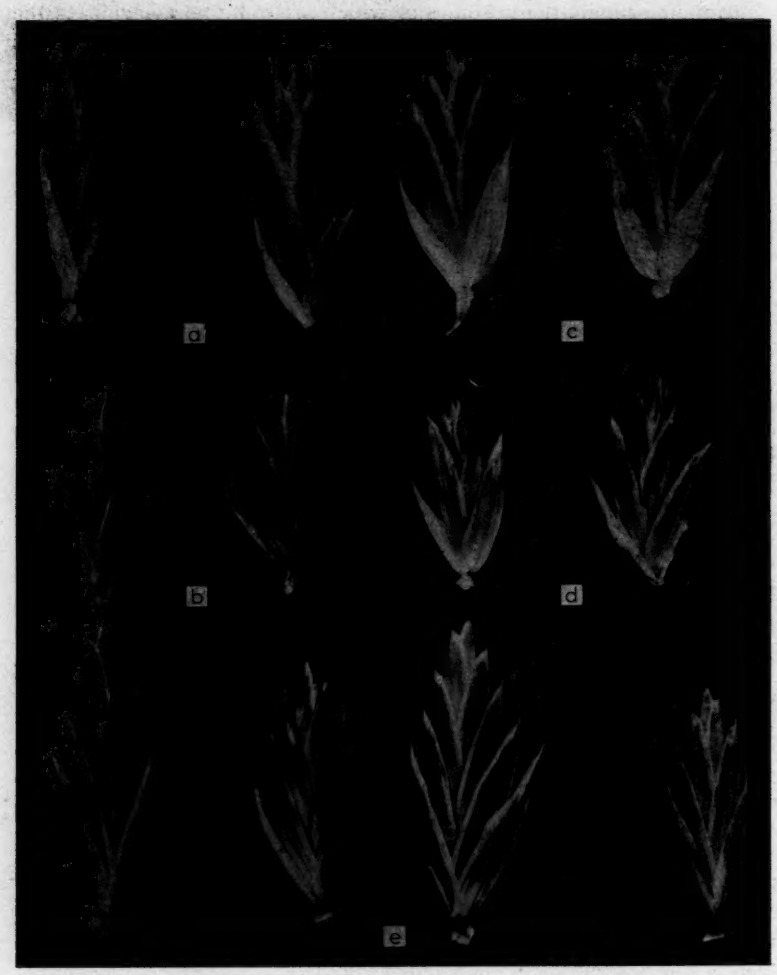

Fig. 2. Spikelets of the *D. glomerata* twins and of *D. Aschersoniana*. — Fig. 2 *a*, two spikelets of the normal twin 41 a ($2n = 28$); *b*, two spikelets of the corresponding haploid 41 b ($2n = 14$); *c*, two spikelets of the normal twin 1060 a ($2n = 28$); *d*, two spikelets of the corresponding haploid 1060 b ($2n = 14$); *e*, spikelets of four different biotypes of *D. Aschersoniana*. The spikelets of haploid *glomerata* (*b* and *d*) are similar to those of *Aschersoniana* (*e*) with regard to the low degree of hairiness and the short acumens.

discussion is no true *Aschersoniana* in morphology it resembles this species in several characters.

As regards meiosis the haploid was rather disappointing. It was found to be completely sterile, the anthers already being degenerated at an early stage before meiosis. Repeated attempts were made to find better anthers, but the result was always quite negative. Thus, sterility in this haploid is absolute and typically diplontic.

Better results were obtained with the other haploid twin plant available (No. 41 b). This haploid is much more vigorous than the previous one and the anthers are better developed. However, in relation to its normal sister twin (No. 41 a), having $2n = 28$, the haploid 41 b has also rather reduced vegetative dimensions. Habitually it is similar

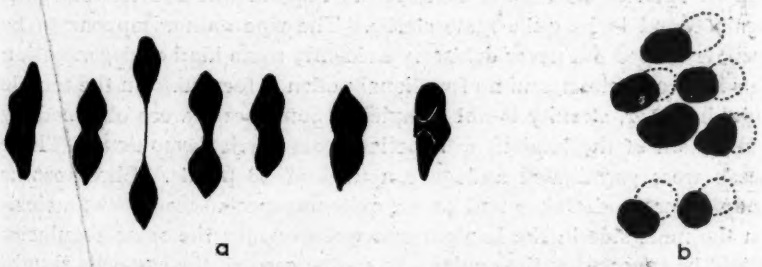

Fig. 3. First metaphase with seven bivalents in haploid *D. glomerata* (the twin plant 41 b). — Fig. 3 *a*, side view (separately drawn); Fig. 3 *b*, polar view. (Aceto-carmine preparations.)

to, but not identical with, *D. Aschersoniana*. It differs from this species by having relatively *glomerata*-like panicles and small spikelets and by having somewhat scabrous leaf sheaths and relatively dark green leaves. All these characters, however, were more pronounced in the normal sister twin (41 a). As regards the morphological details of the spikelets a rather striking similarity to *D. Aschersoniana* was again observed. Just as in the other haploid (1060 b), the spikelets were less hairy and had shorter acumens than in the normal sister twin (Fig. 2). In the present case, however, this reduction, though quite clear, was less pronounced than in the haploid first described.

The good anther development in the second haploid allowed studies of meiosis. Already from the first acetocarmine preparations it was evident that meiosis in this haploid was of just the same type as in *D. Aschersoniana*, the 14 chromosomes regularly forming seven bivalents at diakinesis and first metaphase (Fig. 3). This regularity, which was ex-

pected on account of the autotetraploid constitution of *D. glomerata*, was also verified by a number of slides with sections stained with gentian violet. Exact counts of the chiasma frequency have not yet been undertaken, but from the preliminary observations it is already clear that this frequency must be about the same as in *Aschersoniana*. At any rate, ring bivalents are quite frequent and non-conjunction is rare. Only in a few per cent of the cells the configuration $6_{II} + 2_{I}$, instead of 7_{II}, was observed. First anaphase appears to be quite normal, and also regular second divisions were observed. In the tetraploid sister twin meiosis was found to be of the usual type in *D. glomerata*, being characterized by the presence of a high frequency of quadrivalents (cf. MÜNTZING, 1937 b).

In spite of its regular meiosis the haploid under discussion was again found to be quite male sterile. The ripe anthers appear to be well developed but never dehisce. Evidently some kind of degeneration sets in after meiosis, and no functional pollen is formed. On the female side, however, sterility is not complete. Some seeds were obtained by pollination of the haploid with pollen from *D. Aschersoniana*. These seeds were germinated and gave a total of 10 plants. Chromosome counts were undertaken and gave a quite unexpected result. As meiosis on the male side in the haploid was quite regular the same regularity might be expected in the ovules. In such a case all the egg cells should have 7 chromosomes, and after fertilization with *Aschersoniana* pollen, also having 7 chromosomes, the result should be hybrids between *Aschersoniana* and haploid *glomerata* having $2n = 14$. However, of the ten plants examined eight were triploid, having $2n = 21$, one plant had $2n = \pm 24$, and one plant was tetrapolid ($2n = 28$).

This looks rather like the result of a pollination with *glomerata* pollen ($n = 14$) instead of *Aschersoniana* pollen, and the cross certainly must be repeated in order to exclude this possibility of an experimental error. This repetition has been delayed by the recent very hard winters, which almost killed our haploids. Pending this control another explanation may be suggested. It is possible that in the haploid plant meiosis, though quite regular in the p. m. c., is characterized by asynapsis in the ovules. In such a case most functional gametes will be unreduced and give rise to triploids after fertilization by male gametes with seven chromosomes.

In order to elucidate further the genotypical relationship between *D. Aschersoniana* and *glomerata* it would, indeed, be valuable to obtain diploid hybrids between haploid *glomerata* and *Aschersoniana*. Another

line of approach is to produce tetraploid *D. Aschersoniana* for comparison and hybridization with *glomerata*. Work of this kind is now under way with the help of colchicine.

Finally, it remains to consider the fact that the two haploids of *glomerata* obtained were both rather poor but conspicuously different with regard to vigour as well as fertility. Such striking differences between haploid individuals of the same species have also been observed in other allogamous species. Thus, of the three haploids of *Phleum pratense* obtained by the twin method (MÜNTZING, 1938 a, Table 1) one plant could only be kept alive for one year, and of the surviving two haploids one individual is much more vigorous than the other one (cf. NORDENSKIÖLD, 1941, Figs. 1—2). In *Secale cereale* a total of six different haploids have been observed at Svalöf during the last five years. The first one of these was exceedingly weak (MÜNTZING, 1937 c) and died before maturity; all of the other haploids produced ears, but represented different degrees of vigour (NORDENSKIÖLD, 1939; LEVAN, 1942).

There is reason to believe that the variable and, on an average, poor vigour of the haploids in *Dactylis*, *Phleum* and *Secale* is correlated to the fact that these allogamous species suffer from inbreeding. In their chromosomes there are probably numerous weak points — recessive mutations or structural chromosome alterations — which have no effect in the populations, as the uniting gametes carry different sets of weak points. These weak points, however, will be effective after inbreeding owing to homozygosity. They should also have an effect in haploids, which lack the normal alleles of the weak points. The fact that the haploids really have a more or less reduced vigour and a more or less pronounced degree of diplontic sterility may, indeed, be regarded as a confirmation of the view that inbreeding degeneration is caused by the occurrence of numerous deleterious recessive factors present in the allogamous populations (cf. MÜNTZING, 1938 b, 1939).

The situation in *Dactylis glomerata* is complicated by the fact that this species is an autotetraploid, forming quadrivalents at meiosis. Thus, there are seven sets of four homologous chromosomes. Theoretically, a deleterious factor *a* may be present in one to four of these chromosomes, giving the combinations *AAAa*, *AAaa*, *Aaaa* and *aaaa*, *A* being the normal allele of *a*. The combination *aaaa* would not occur in the population but might be produced by inbreeding. As to the other combinations, it is a question whether only plants of the type *AAAa* will occur in the populations or also *AAaa* and *Aaaa* individuals. The poor

constitution of both the haploid *glomerata* plants obtained is evidence in favour of the latter alternative. If the mother plants had been *AAAa* the haploids would have been relatively vigorous *AA* or *Aa* plants (disregarding the slight possibility of *aa* individuals arising from *AAAa*). As the haploids were poor *aa* plants the mother individuals must have been either duplex or triplex for the deleterious factor (*AAaa* or *Aaaa*). It is probable, of course, that the poor properties of the haploids is caused by several factors in different sets of four chromosomes. Nevertheless, the result strongly indicates that deleterious factors are rather frequent in the *glomerata* chromosomes, and that the same factor is often present in more than one of the four homologues. This will not do any harm in the populations, but when the chromosome number is suddenly halved the protective effect of the normal alleles may be removed.

It would be interesting to produce a larger number of haploid *glomerata* plants and this is only a question of time. Some of these haploids should have a better fertility and vigour than those already obtained. The study of such plants may evidently to a certain degree elucidate the causes of inbreeding degeneration in *Dactylis glomerata* as well as the genotypical relationship between *D. glomerata* and *Aschersoniana*.

LITERATURE CITED.

1. LEVAN, A. 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas XXVIII, pp. 177—211.
2. MÜNTZING, A. 1937 a. Polyploidy from twin seedlings. — Cytologia, FUJII Jubilee Volume, pp. 211—227.
3. — 1937 b. The effects of chromosomal variation in *Dactylis*. — Hereditas XXIII, pp. 113—235.
4. — 1937 c. Note on a haploid rye plant. — Hereditas XXIII, pp. 401—404.
5. — 1938 a. Note on heteroploid twin plants from eleven genera. — Hereditas XXIV, pp. 487—491.
6. — 1938 b. Sterility and chromosome pairing in intraspecific *Galeopsis* hybrids. — Hereditas XXIV, pp. 117—188.
7. — 1939. Chromosomenaberrationen bei Pflanzen und ihre genetische Wirkung. — Zschr. f. ind. Abst.- u. Vererb.-lehre, Bd. 76, S. 323—350.
8. NORDENSKIÖLD, H. 1939. Studies of a haploid rye plant. — Hereditas XXV, pp. 204—210.
9. — 1941. Cytological studies in triploid *Phleum*. — Botaniska Notiser (Lund), pp. 12—32.

AN INTERSPECIFIC TRANSLOCATION CAUSING CHANGED SEGREGATION RATIOS

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THE species hybrid *Godetia Whitneyi* × *deflexa* was produced by Dr. GUNNAR HIORTH, of the Agricultural College at Ås, Norway. This hybrid shows complete asyndesis; it has 16 I (HÅKANSSON, 1941). By means of repeated back-crossings with *Whitneyi* HIORTH has obtained a type that more closely resembles *G. Whitneyi* but has red foliar nerves like *G. deflexa*. The new type also possesses certain characters that are reminiscent of trisomics. The red nervature of the leaves is produced by a dominant gene (*R*), but self-fertilization of red-nerved plants yields only 9 % red-nerved members in the progeny; reciprocal crosses with *Whitneyi* also gave only a low percentage of red-nerved plants. Cytological studies of a number of plants belonging to the red-nerved type showed that they had 15 chromosomes; one of these always behaved as a univalent during the first metaphase and was very often eliminated during meiosis (HÅKANSSON, l. c.). It is evident that the extra chromosome that cannot pair with any of the others is a *deflexa* chromosome, the great deficiency in red-nerved offspring being due to this elimination.

In his attempts to install the gene for red foliar nerves in *Whitneyi* HIORTH has also employed the method of X-raying the pollen of red-nerved plants. By this means a family, S 929—1942, has now been obtained in which the frequency of red-nerved plants after selfing has risen to almost 50 %. The pollen of the red-nerved grandfather of S 929 had been treated with X-rays and the red-nerved plants obtained had then been selfed; 8 segregated in 25 red-nerved: 436 normal plants, 1 segregated in 55 red-nerved: 64 normal plants. Red-nerved plants in S 929 have not a trisomic habit, having a different appearance and resembling a *Whitneyi* race called Bremen. This information has been communicated to me by letter from Dr. HIORTH (see also HIORTH, 1942).

I have examined six plants of S 929—1942. Fixings of two plants did not contain meiotic stages, but the somatic divisions in young petals could nevertheless be studied. Meiosis could be studied in four plants.

The new red-nerved plants were found to have 14 chromosomes, and during the first metaphase they form seven II.

A pronounced terminalization of chiasmata takes place in *G. Whitneyi*, although the completed bivalents may have an interstitial chiasma on one side of the centromere, quite close to the end of the chromosomes. There is thus formed quite a short arm of somewhat varying length. The number of interstitial chiasmata of this kind varies; sometimes there is one in the bivalent, mostly there is none; some *Whitneyi* types frequently have interstitial chiasmata, others rarely.

In the new red-nerved form, however, only six of the bivalents

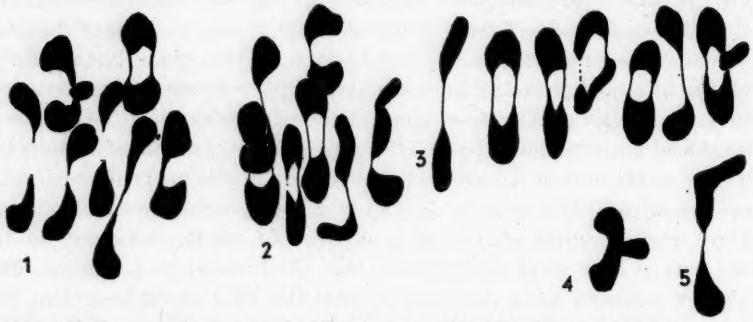

Fig. 1: Early metaphase 1, the translocation bivalent has a lateral arm. — Fig. 2: Early metaphase 1, the translocation bivalent is heteromorphic. — Fig. 3: Bivalents separately drawn. — Figs. 4 and 5: Equational and reductional translocation bivalent. — × 3800.

have developed in this way. One bivalent, however, has a rather long lateral arm as a constant feature (Figs. 1 and 3). In the PMCs in which this arm is missing, a heteromorphic bivalent is seen (Fig. 2), the paired chromosomes having different lengths and the portion corresponding to the lateral arm being seated here at the end of one of the chromosomes. Thus, these PMCs exhibit no fully developed chiasma between the centromere and the extension of the chromosome, and the two chromosomes are arranged reductionally. The extension appears, however, as a lateral arm when a chiasma is formed on its side of the centromere. The chiasma formed cannot be terminalized completely, only up to the point where the new chromosome fragment is inserted.

A similar bivalent has been observed by me in *G. Whitneyi* (HÅKANSSON, 1940, pp. 26—29). It was in a plant (a mutant) that had developed after X-ray treatment of pollen. It was interpreted as a translocation, a fragment of one chromosome having attached itself

to or near the end of another. The lateral arm was divided during the first anaphase (l. c., Fig. 12 *g* and *h*). At the first division, thus, equational division most frequently takes place, more rarely reductional division of the heteromorphic bivalent. A translocation of this type was previously known in *Datura Stramonium* (BERGNER and BLAKESLEE, 1934). Further cases of this kind of simple translocation are found in the last-mentioned species (BERGNER, 1939). They have all arisen after radiation or X-raying.

In the previously examined translocation plants the heteromorphic bivalent often took part in the formation of a trivalent or a quadrivalent. These cases involved a translocation within the same chromosome set, and in the genome were portions homologous to the translocated fragment. Multivalent formation was never observed in the red-nerved translocation. Evidently the translocated portion has no homologous part among the bivalents. It must therefore have come from the *deflexa* chromosome. This explains the altered transmission of the red-nerved character. Under the action of the X-rays a rather large fragment (about the length of the lateral arm) of the *deflexa* chromosome containing the gene *R* has broken off and become attached to the end of a *Whitneyi* chromosome. The new red-nerved type has this altered chromosome, while the rest of the *deflexa* chromosome is missing. Now that the *R* gene is no longer in a univalent, it is not eliminated, but a segregating ratio is obtained that approaches the normal.

The new red-nerved type can hardly be said to constitute a cytologically favourable object of study. The first metaphase is easy to study, but the second division is difficult to get well fixed, and therefore the later behaviour of the translocation chromosome has not admitted of being closely followed.

Whereas in the cases cited a translocation within the same genome is concerned, in the formation of the new red-nerved type an interspecific translocation has occurred. Such can therefore take place and give rise to an exactly similar bivalent, in which the translocated portion juts out as a lateral arm because the terminalization has been arrested owing to change of homology (according to DARLINGTON). In a species hybrid containing two or more quite different genomes, e. g. an allotetraploid, that has arisen from an asyndetic diploid primary hybrid, there can probably take place translocations or exchanges between the different genomes which bring about a levelling out of the differences between them.

So far we do not know if plants homozygous for the translocation are viable or not. If they are unviable the segregation in S 929—1942 shows a close resemblance to the segregation of red-nerved plants of *Oenothera Lamarckiana.*

LITERATURE CITED.

1. BERGNER, A. D. 1939. Chromosome association in *Datura.* — Proc. of the seventh international genetic congress.
2. BERGNER, A. D. and BLAKESLEE, A. F. 1934. Cytology of a translocation of the 1, 2 chromosome in *Datura.* — Bull. Torrey Bot. Club 61.
3. HIORTH, G. 1942. Eine Translokation zwischen einen *Godetia Whitneyi*- und einen *G. deflexa*-Chromosom. — Zschr. f. ind. Abst.- und Vererbungslehre, Bd. 80.
4. HÅKANSSON, A. 1940. Die Meiosis bei verschiedenen Mutanten von *Godetia Whitneyi.* — Lunds Univ. Årsskrift, N. F. Avd. 2, Bd. 36, Nr. 9.
5. — 1941. Zytologische Beobachtungen an Kreuzungen zwischen *Godetia deflexa* und *G. Whitneyi.* — Botaniska Notiser.

THE SIGNIFICANCE OF DIFFERENCES IN THE DISTRIBUTION OF DIPLOIDS AND POLYPLOIDS

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I. INTRODUCTION.

IT is a well-known fact that closely related diploid and polyploid species almost always show differences in distribution. In the great majority of the cases so far observed the polyploids are to be found in a more extreme climate than their diploid relatives (cf. MÜNTZING, 1936). As a direct result of this phenomenon the percentage of polyploid species is considered to increase from south to north as well as from the lowlands to the alpine regions. Such an increase has been previously presumed by HAGERUP (1931) and clearly demonstrated by TISCHLER (1935), FLOVIK (1940) and others, even if none of these workers have used fully adequate statistical methods to show the nature of the differences in polyploidy within the different areas.

The only extensive paper on the differences in the frequency of polyploids within the floras of distinct regions was published by TISCHLER (1935). His investigations were made on the floras of Sicily, Schleswig-Holstein, the Faeroes and Iceland, and simple statistical calculations showed clear differences between the four countries. He was able to demonstrate clearly that in Schleswig-Holstein the percentage of polyploids was much higher between plants with a northern or circumpolar distribution than between plants with a distribution towards the south, and all his tables show higher percentages of polyploids within the monocotyledons than within the dicotyledons.

In listing the chromosome numbers known within the species of Scandinavian Spermatophyta (LÖVE and LÖVE, 1942) the present writers made some preliminary observations on the differences in polyploidy within the Scandinavian countries. In order to test the statistical significance of these differences we undertook a review of the frequency of polyploidy, partly within the Scandinavian countries and partly within some other flora regions. The results of these calculations are recorded in the present paper.

II. THE MATERIAL.

The data used for the investigation were obtained from the papers and persons referred to in Table 1. Of these data the Scandinavian as well as the Icelandic and Faeroeic numbers are calculated by the present writers on the same principles as given by LÖVE and LÖVE (1942). The taxonomic treatment of the material from Scandinavia is the same as that employed by HYLANDER (1941), with the few modifications made by us in the list of the chromosome numbers of Scandinavian plant species (l. c.). The lists of the Icelandic and Faeroeic plants were made by the present writers with the aid of the Icelandic Flora by STEFANSSON (1924) and the Flora of Iceland and the Faeroes by OSTENFELD and GRÖNTVED (1934) with additions from STEINDORSSON (1934), DAVIDSSON (1937) and LÖVE (1941). As the limitation of the species of these two countries is made in full accordance with that of HYLANDER (l. c.) and LÖVE and LÖVE (l. c.) for the Scandinavian countries, the numbers from these six Nordic countries used for the present calculations are fully comparable. The distinctions between diploids and polyploids were made on the same grounds as used by us (l. c.), and in cases when both diploid and polyploid numbers are recorded for the species the decision was made in favour of the diploids, since these species were included among the diploids. Therefore, the number of polyploids may actually be somewhat higher in these countries. The numbers for Schleswig-Holstein were kindly sent to us by Professor Dr. G. TISCHLER, Kiel, and were calculated on almost the same principles as the numbers from Scandinavia. Except for the genera *Rubus*, *Taraxacum* and *Hieracium* this region is fully comparable with the Scandinavian ones. The genus *Rubus* is included in the Scandinavian numbers as 62 polyploids (most from Denmark and Sweden) and 2 diploids, but as this polymorphic genus has not been treated in such taxonomic detail in Schleswig-Holstein as in Scandinavia, it is included in the number from that region as only one polyploid species. The genera *Taraxacum* and *Hieracium* are entirely excluded from the numbers counted by the present writers, but in the calculations from Schleswig-Holstein they are included as 9 collective species of *Hieracium* and 2 of *Taraxacum*. These differences in the treatment, however, should only be responsible for a small part of the differences in polyploidy found between Schleswig-Holstein and the Scandinavian countries, the differences being mainly found within the monocotyledons, as will be explained later (cf. Table 4).

TABLE 1. *The percentage of polyploids within different regions.*

	Km²	latitude	% determ.	Perc. of polyploids monoc.	dic.	total	Perc. monoc. of det. angsp.	According to:
Timbuctoo	—	ca. 17° N.	21,0[1]	67	31	37	10,38	HAGERUP, 1931.
Sicily	25 000	36—39° N.	37,6	51,5	32,8	37,0	22,87	TISCHLER, 1942 (in lit.)
Schleswig-Holstein..	20 000	54—55° N.	92,0	63,1	43,5	48,7	26,70	TISCHLER, 1942 (in lit.)
Denmark	44 000	54 1/2—58° N.	87,7	70,1	45,8	52,1	25,80	LÖVE and LÖVE, 1942.
Sweden	449 000	55 1/2—69° N.	81,5	74,6	45,9	53,7	27,08	LÖVE and LÖVE, 1942.
Norway	323 000	58—71° N.	83,6	74,3	45,9	54,1	28,96	LÖVE and LÖVE, 1942.
Finland	383 000	60—70° N.	80,7	77,4	45,4	54,6	28,79	LÖVE and LÖVE, 1942.
Faeroes	1 400	ca. 62° N.	85,0	76,1	51,7	60,7	36,82	LÖVE and LÖVE (unpubl.)
Iceland	106 000	63 1/2—66 1/2° N.	80,0	84,3	53,4	63,5	32,58	LÖVE and LÖVE (unpubl.)
Spitzbergen	63 000	77—81° N.	—	95,2	68,4	77,4	33,87	FLOVIK, 1940.
Alpine climates								
Caucasus	—	41—44° N.	—	—	—	50	—	SOKOLOVSKAJA and STRELKOVA, 1940.
Áltai	—	49—50° N.	—	—	—	65	—	SOKOLOVSKAJA and STRELKOVA, 1938.
Pamir	—	ca. 37° N.	—	—	—	85	—	SOKOLOVSKAJA and STRELKOVA, 1938.
Halophytes								
Schleswig-Holstein..	—	54—55° N.	100,0	—	—	55,6	—	H. D. WULFF, 1937.
Roumania	—	44—46° N.	—	—	—	26,3	—	TARNAVSCHI, 1938.

[1] Cf. HAGERUP, 1930.

The figures from Sicily were calculated and kindly sent to us by Professor TISCHLER. Unfortunately, the number of species with known chromosome numbers is relatively low from this region, and especially the typical Mediterranean species are still unknown. The number of polyploids from this area is, therefore, certainly somewhat too large, since a much larger number of the cytologically unknown species of Liliaceae, Orchidaceae, Cistaceae, Umbelliferae, Labiatae and Compositae very probably belong to the diploids rather than to the polyploids, similar to the cases already known within these families.

The data used from Timbuctoo (cf. HAGERUP, 1930, 1931) are much older than the rest, but as they may be of some interest we considered it appropriate to include them in the present calculations.

The numbers from Spitzbergen are taken from the paper by FLOVIK (1940). Even if they do not include some of the larger families as, e. g., Cyperaceae and Juncaceae, they may be of great value for an investigation like the present one.

III. DESCRIPTION OF RESULTS.

a. *The percentages.* — The frequency of polyploids within the different regions investigated in the present paper is shown in Table 1. Although the chromosomal composition of the Angiosperms is not known in the same proportion within all the areas studied, the table is quite instructive.

The table shows *a decided increase in the percentages of polyploids with an increase in latitude*, i. e. the same state of affairs as was previously shown by TISCHLER (1935). It also shows *a distinctly higher percentage of polyploid monocotyledons than of dicotyledons* within all the regions studied. The frequency of polyploids is also somewhat higher on the Faeroes and Iceland than, for instance, in Finland, even if these regions lie at almost the same latitude. The possible cause of this will be discussed later on.

The percentage of monocotyledons, too, increases with an increase in latitude and in more extreme climates, a well-known fact of great interest to the cyto-ecologist, as polyploidy is known to be very frequent within at least some of the larger families of monocots, such as Cyperaceae and Gramineae, and species belonging to these families are very common far to the north and in alpine regions.

As the differences between the regions are often rather small, a

TABLE 2. *The number of cytologically determined Angiosperms within some regions at different latitudes.*

	Monocotyledons diploid	Monocotyledons polyploid	Dicotyledons diploid	Dicotyledons polyploid	Total diploid	Total polyploid	χ^2 for frequencies of polyploids within monocots and dicots
Timbuctoo	1	2	18	8	19	10	—
Sicily	98	104	458	223	556	327	*23,460*
Schleswig-Holstein	97	166	408	314	505	480	*29,728*
Denmark	84	197	438	370	522	567	*49,388*
Sweden	88	258	505	429	593	687	*83,256*
Norway	79	228	407	346	486	574	*70,437*
Finland	64	219	382	318	446	537	*84,937*
Faeroes	21	67	73	78	94	145	*13,964*
Iceland	15	86	97	111	113	197	*59,343*
Spitzbergen	1	20	13	28	14	48	*57,676*

close statistical analysis of the empirical data was made. The data for the first part of the analysis are given in Table 2.

b. *Statistical methods.* — The most useful statistical method for a study like the present one is the χ^2 analysis (cf. BONNIER and TEDIN, 1940; FISHER, 1936). For these calculations the χ^2 values were obtained by the following method:

The number of diploid species in the region, or group X, is marked with a, the number of polyploid species is b, and in the region, or group Y, the same values are c and d respectively. These values are compiled into a table like the following

X	a	b	$a+b$
Y	c	d	$c+d$
	$a+c$	$b+d$	$a+b+c+d$

and the value of χ^2 is obtained by the formula

$$\chi^2 = \frac{[(a \times d) - (c \times b)]^2 \times (a+b+c+d)}{(a+c)\ (b+d)\ (a+b)\ (c+d)}$$

The significance of the values of χ^2 $(= P)$ for one degree of freedom is, according to BONNIER and TEDIN (1940):

$P =$	0,99	0,98	0,95	0,90	0,80	0,70	0,50	0,30	0,20	0,10
$\chi^2 =$	*0,000*	*0,001*	*0,004*	*0,016*	*0,064*	*0,148*	*0,455*	*1,074*	*1,642*	*2,706*

0,05	0,02	0,01	0,001
3,841	*5,412*	*6,635*	*10,827*

When the number of individuals is low in one or both groups the value of χ^2 may not give a clear significance. With the same proportionality, however, χ^2 increases with an increase in the number of individuals.

In cases in which χ^2 gives a P equal to or lower than 0,05 the figures in the tables are printed in italics.

c. *Monocotyledons contra dicotyledons.* — In order to decide whether the differences in frequency of polyploidy between the monocotyledons and dicotyledons within the different regions given in Table 1 were statistically significant, the χ^2 values for polyploid and diploid monocots and polyploid and diploid dicots were determined by the above-mentioned method. The results are given in Table 2. The differences are highly significant in all the regions but Timbuctoo, where the number of individuals is too low to give a satisfactory result. Therefore, *it may be regarded as statistically demonstrated that the monocotyledons show a higher degree of polyploidy than the dicotyledons* within the regions studied.

d. *Frequencies of polyploids within different regions.* — Table 3 gives all the values of χ^2 for the differences in frequency of diploids and polyploids between the ten regions. Even if the number of species

TABLE 3. *Frequencies of polyploids within the Angiosperms (χ^2 values).*

	Timbuctoo	Sicily	Schleswig-Holstein	Denmark	Sweden	Norway	Finland	Faeroes	Iceland
Sicily	0,078	—	—	—	—	—	—	—	—
Schleswig-Holstein	2,290	*25,966*	—	—	—	—	—	—	—
Denmark	*4,289*	*44,467*	2,301	—	—	—	—	—	—
Sweden	*4,194*	*58,093*	*5,440*	0,608	—	—	—	—	—
Norway	*4,390*	*56,762*	*6,005*	0,987	0,005	—	—	—	—
Finland	*4,603*	*57,924*	*6,853*	1,362	0,220	0,046	—	—	—
Faeroes	7,272	*43,118*	*10,970*	*5,833*	*3,980*	3,353	2,844	—	—
Iceland	*9,422*	*65,494*	*20,570*	*12,834*	*9,860*	*8,608*	*7,640*	0,476	—
Spitzbergen	*15,759*	*39,477*	*19,203*	*15,153*	*13,462*	*15,296*	*12,293*	*6,003*	*4,421*

known from Timbuctoo is very low, the differences between this region and Schleswig-Holstein are almost statistically significant and a relativ-

ely high degree of significance is found between Timbuctoo and the Faeroes, Iceland and Spitzbergen. The differences between Timbuctoo and Sicily are not significant, partly due to the relatively low difference in latitude and partly to the low number of observations from Timbuctoo. Mainly, however, the low significance between these two regions may be due to the phenomenon, pointed out by HAGERUP (1931), that the extreme climate of the desert at Timbuctoo will probably be better tolerated by polyploid than diploid species, giving an unexpectedly high frequency of polyploids at this low latitude.

All the values in Table 3 show a clear tendency to an increase of χ^2 with an increase in distance between the regions. Regions lying close to one another, as for instance the Scandinavian countries, show no significant differences, but even if only a very low value of χ^2 is found between Schleswig-Holstein and Denmark and Denmark and Finland it is rather significant between Schleswig-Holstein and Sweden. Summarizing the results given in Table 3 it is to be emphasized that *a general tendency in favour of the hypothesis of* TISCHLER (1935) *is found between all the countries, and the statistical significance of the differences increases proportionally to the increase in the differences in latitude between each of the regions.* As the value of χ^2 increases with the number of observations — the proportion being constant — it should, perhaps, be pointed out that the increase of χ^2 with increasing latitude is *not* caused by an increase in numbers with increasing latitude.

e. *Polyploidy within the monocotyledons and dicotyledons.* — As mentioned above, significant differences were found between the frequency of polyploids within the monocotyledons and dicotyledons, the latter always showing a lower percentage of polyploidy. It is also assumed that the percentage of monocotyledons is higher in arctic regions than in temperate climates. Therefore, it is of special interest to investigate whether the differences are greater in one of these groups than in the other in the regions studied. The results of calculations of χ^2 for the monocotyledons and dicotyledons separately are given in Table 4.

As might be expected, the values of χ^2 show on the whole the same increasing tendency with an increase in distance between the areas, both in the dicots and the monocots. The comparisons between, e. g., Sicily and the regions to the north show that, even if they all give very significant differences, the χ^2 values for the monocotyledons are somewhat lower than those for the dicotyledons for some of the regions. Comparing, however, the four Scandinavian countries and Schleswig-

TABLE 4. *Frequencies of polyploids within the monocotyledons and the dicotyledons (χ^2 values).*

		Monocotyledons							
	Timbuctoo	Sicily	Schl.-Holst.	Denmark	Sweden	Norway	Finland	Faeroes	Iceland	Spitzbergen
Dicotyledons										
Timbuctoo	×	0,272	0,016	0,024	0,097	0,089	0,194	0,141	0,764	2,805
Sicily	0,044	×	*6,349*	*17,354*	*30,304*	*27,879*	*35,224*	*15,394*	*32,628*	*14,751*
Schl.-Holst.	1,656	*17,124*	×	2,958	*9,260*	*8,240*	*13,345*	*5,007*	*16,627*	*8,877*
Denmark	2,294	*26,245*	0,817	×	1,549	1,267	*3,859*	1,196	*8,755*	*6,102*
Sweden	2,344	*28,443*	0,981	0,374	×	0,007	0,674	0,001	*4,996*	*4,605*
Norway	2,836	*26,046*	0,901	0,611	0,001	×	0,779	0,126	*5,199*	*4,687*
Finland	2,170	*23,301*	0,540	0,709	0,040	0,089	×	0,059	2,745	3,706
Faeroes	*3,873*	*19,140*	3,985	1,767	1,710	1,644	1,935	×	2,477	*3,841*
Iceland	*4,721*	*28,881*	*6,345*	*7,378*	3,771	3,593	*4,052*	0,102	×	1,553
Spitzbergen	*9,011*	*21,546*	*9,649*	*7,932*	*7,886*	*7,790*	*8,134*	3,009	3,004	×

Holstein it will be seen that no significant differences are found between the frequencies of polyploids within the dicotyledons but that a relatively low significance is obtained between the monocotyledons of Schleswig-Holstein and Denmark and rather significant values are obtained for the differences between Schleswig-Holstein and Sweden, Norway and Finland. This fact may possibly be explained as a result of the higher frequency of polyploid species of monocotyledons in the more northern floras.

It will also be observed when comparing the differences between, for instance, Iceland and Spitzbergen that there are much greater differences between the dicotyledons than between the monocotyledons of these countries, contrary to the above-mentioned results from the five regions in North-Europe.

Table 4 clearly shows that even if the polyploidy is more frequent among the monocotyledons than among the dicotyledons its frequency within a distinct region increases with an increase in latitude in both the groups of Angiosperms. It also seems as if it would increase somewhat more within the monocotyledons than within the dicotyledons at higher latitudes (Schleswig-Holstein : Denmark, Denmark : Finland), even if the number of species studied from Spitzbergen is far too low to give any comparable result.

f. *Polyploidy within the same families.* — As a number of species and families of Angiosperms is only

TABLE 5. *Number of species determined within the same families.*

	Denmark		Sweden		Norway		Finland		Faeroes		Iceland		Spitzbergen	
	dipl.	polypl.	dipl.	polypl.	dipl.	polypl.	dipl.	polypl.	dipl.	polypl.	dipl.	polypl.	dipl.	polypl.
Gramineae	43	58	47	77	38	68	29	69	8	23	8	29	1	20
Polygonaceae ...	6	18	8	19	7	17	6	19	2	8	2	8	1	1
Caryophyllaceae	37	15	42	22	34	21	34	17	7	8	12	9	2	3
Ranunculaceae ..	16	17	26	24	19	18	22	22	3	6	5	5	2	4
Papaveraceae ...	6	2	6	3	3	5	0	4	0	1	0	1	0	1
Cruciferae	27	30	34	34	31	29	24	25	3	5	6	10	1	4
Saxifragaceae ...	6	4	12	10	11	14	11	10	3	4	5	11	3	7
Rosaceae	16	84	24	80	14	52	16	46	3	10	5	10	2	1
Empetraceae	1	0	1	1	1	1	1	1	1	1	1	1	0	1
Ericaceae	13	6	17	8	18	8	16	8	6	1	10	4	1	0
Campanulaceae..	6	3	6	3	7	3	6	2	0	1	0	1	0	1
Compositae	60	31	61	36	51	30	43	33	8	3	7	5	0	4
Total:	237	268	284	317	234	266	208	256	44	71	61	94	13	47
% polyploids: ..	53,1		52,7		53,2		55,2		61,7		60,6		78,3	
Total monocots:	43	58	47	77	38	68	29	69	8	23	8	29	1	20
% polyploids: ..	57,4		62,1		63,0		70,4		74,1		78,3		95,2	
Total dicots: ...	194	210	237	240	196	198	179	187	36	48	53	65	12	27
% polyploids: ..	52,0		50,3		50,3		51,1		57,1		55,1		69,2	

found within some of the regions studied, the results mentioned above show nothing certain about the adaptation of polyploids, as, theoretically, they may depend only on differences in distribution of families and the different number of species as a whole within each of the regions. In order to see whether the same differences as were found between the six Nordic countries and Spitzbergen in Tables 3—4 would also be found if only the known species of the *same* families were calculated, we constructed a list over the polyploidy within the different families known from all the seven regions. This list is found in Table 5. Only families with representatives in *all* the countries were included in the calculations.

Comparing the percentages of polyploids in Table 5 and Table 1, it is evident that *the frequencies of polyploids within the whole flora is almost the same as within the families studied.* The percentages are somewhat lower for Sweden, Norway and Iceland in Table 5 than in Table 1, but somewhat higher in the other countries. The values of χ^2 given in Table 6 show the same general tendency as those in Table 3, even if significant values are not obtained, except for Spitzbergen contra all the other countries.

TABLE 6. *Frequencies of polyploids within the same families of Angiosperms (χ^2 values).*

	Denmark	Sweden	Norway	Finland	Faeroes	Iceland
Sweden	0,011	—	—	—	—	—
Norway	0,001	0,022	—	—	—	—
Finland	0,430	1,178	0,377	—	—	—
Faeroes	3,299	3,145	2,752	1,616	—	—
Iceland	2,748	3,099	2,649	1,416	0,033	—
Spitzbergen ...	*13,875*	*14,435*	*13,726*	*11,686*	*4,943*	5,995

A glance at the values for the monocotyledons and dicotyledons in Tables 1 and 5 makes it clear that the values for the same families (Table 5) are somewhat higher for the dicots and much lower for the monocots than in Table 1. Therefore, we analysed these groups separately, and the values of χ^2 calculated from the figures in Table 5 are given in Table 7. Within the dicotyledons practically no significant differences are found between the six Nordic countries, even if the tendency is the same as found before. The values found for the dicots from the Faeroes, however, were somewhat higher than those for Iceland, indicating a somewhat higher frequency of polyploids within the families studied from the Faeroes. Both these countries show a higher frequency of polyploids than expected, if only the present

climatic conditions were the responsible factors. A statistically significant difference is met with between the dicotyledons of the Scandinavian countries and Spitzbergen only.

Analysing the differences in polyploidy within the monocotyledons (= Gramineae), the results were strikingly different from those for the dicotyledons (cf. Table 7). Here the percentage of polyploids increases clearly from south to north in the Scandinavian countries and Spitzbergen as well as on the Faeroes and Iceland. Statistically rather significant values are obtained for Denmark and Iceland and the Faeroes and Spitzbergen, and an almost significant value is obtained

TABLE 7. *Frequencies of polyploids within the same families of the monocotyledons and dicotyledons (χ^2 values).*

		Monocotyledons					
	Denmark	Sweden	Norway	Finland	Faeroes	Iceland	Spitzbergen
Dicotyledons							
Denmark .	×	0,506	0,982	3,630	2,812	*5,102*	*10,753*
Sweden ...	0,242	×	2,289	2,519	1,585	3,358	*9,029*
Norway ...	0,237	0,001	×	0,903	1,084	2,544	*7,960*
Finland ..	0,060	0,050	0,053	×	0,164	0,857	*5,564*
Faeroes ..	0,743	1,333	1,315	0,951	×	0,164	*3,873*
Iceland ...	0,353	0,861	0,848	0,569	0,084	×	2,601
Spitzbergen	*4,254*	*5,166*	*5,119*	*4,632*	1,635	2,417	×

for the difference between Denmark and Finland and Sweden and Iceland. If the numbers compared had been somewhat higher — the proportion being the same — statistically significant differences would surely have been found between Finland and Sweden as well as between Iceland and Norway and Iceland and Spitzbergen.

g. *Alpine regions.* — According to MÜNTZING (1936) and a great number of workers studying the geographical distribution of diploid and polyploid species of different genera, the polyploid species should be more frequent than the diploids in the more alpine climates. The only data known to the writers on the frequency of polyploidy within alpine regions with climates of different severity are those published by SOKOLOVSKAJA and STRELKOVA (1938, 1940), who studied the alpine regions of Pamir, Altai and Caucasus. According to them, the severest climate and the highest frequency of polyploids are found at Pamir, but Altai, which lies more northerly, and Caucasus, which lies at almost the same latitude as Pamir, have a somewhat milder climate and a lower frequency of polyploids. Unfortunately, these workers do not state the altitude of the localities studied in the different regions nor the size of the regions in square kilometres. Their results, however, are

statistically very significant, as shown in our Table 8. The unexpected low frequency of polyploids in Caucasus is, according to SOKOLOVSKAJA and STRELKOVA (1940), suggested to be the result of a rather great migration from the south, from the regions of the Mediterranean and Asia Minor.

TABLE 8. *Polyploids in alpine climates.*

	Empirical data		χ^2 values	
	diploids	polyploids	Pamir	Altai
Pamir	23	127	×	×
Altai	70	130	*16,991*	×
Caucasus..	82	82	*42,301*	*8,336*

h. *Halophytes.* — TISCHLER (1937) and H. D. WULFF (1937) have shown that the halophytes of Schleswig-Holstein show a somewhat higher percentage of polyploids than the region as a whole. As the number of halophytes is rather low, no significant differences were found with the method used by the present writers, giving the value $\chi^2 = 0{,}195$. The halophytes of Roumania studied by TARNAVSCHI (1938) showed a very much lower percentage of polyploids than those of Schleswig-Holstein. It was suggested that this phenomenon was the result of the difference in latitude between Schleswig-Holstein and Roumania. The present writers included the major data of H. D. WULFF (1937) and TARNAVSCHI (1938) in the calculations (cf. Table 9) and the value of the χ^2 obtained was 9,144, i. e. a statistically very significant

TABLE 9. *Polyploids in halophytous floras.*

	diploids	polyploids
Schleswig-Holstein	29	31
Roumania	28	10
	$\chi^2 =$	*9,144*

difference. It is to be suggested that the differences in polyploidy within the halophytes of the two regions can partly be due to the differences in climate as well as to the fact that the Ice Age never reached the Roumanian coasts. Whether the polyploidy is really more frequent within the halophytes than within other groups of Angiosperms in a certain region can only be statistically demonstrated when material from other parts of, e. g., the Roumanian flora is compared with the halophytous material.

IV. DISCUSSION.

The present investigation was made mainly in order to ascertain whether the differences in percentage of polyploids within different regions discussed by some previous authors were really statistically significant. The general results of the present calculations are in full accordance with the previous investigations, as *the same general tendency to an increase in polyploidy with an increase in latitude was found in all the calculations. It may, therefore, be taken as a statistically demonstrated fact that the theory of the greater hardiness or adaptability of polyploids than of diploids to more severe climate is correct.* Of course, this does not mean that polyploids will in every case be found in more extreme climates than their diploid relatives (cf. e. g. FLOVIK, 1940; ÖSTERGREN, 1942), but in most of the cases the polyploids will follow the general rule.

It may be of special interest to compare the frequency of polyploids with the well-known fact of the preponderance of species in tropical and moderate climates and the low number of species in arctic and antarctic regions. This difference must at least partly be the result of the difference in temperature, i. e. in latitude. No exact statements as to the total number of Angiosperms in the world are to be found in the current literature, but according to DOBZHANSKY (1937) the number in question is estimated at about 133000 species. Of these only about 10 per cent are as yet cytologically known, and only some 30 per cent of the species with chromosome numbers determined show polyploid numbers. Merely a very few papers have as yet been published on the number of species in different parts of the world, but according to E. V. WULFF (1937) about 40000 species of Angiosperms are to be found in the equatorial zone, whereas only about 500—600 species belong to the arctic and antarctic zones. In India about 20000 species are known, but in the arctic parts of Asia only about 200 species occur. As an extreme example of the differences in the number of species at almost the same longitude it may be mentioned that on Ceylon (8—10° N. lat.) there are 3074 Angiosperms, but on Sibiryakov Island (ca. 80° N. lat.) only 62 species have been found, all these statements according to E. V. WULFF (1937).

It must be more than a coincidence that the decrease in number of species is followed by an increase in percentage of polyploids. The primary cause of the low number of plants at higher latitudes is assumed to be the low temperature. According to the majority of detailed studies

on the distribution of closely related polyploid and diploid species (cf. e. g. MÜNTZING, 1936), the polyploids are to be regarded as more hardy than their diploid relatives. In connection with this hypothesis the higher frequency of polyploids in areas with a low number of Angiosperms is assumed to be mainly the result of the relatively greater hardiness or adaptability of the polyploids, so that when more extreme climate causes a decrease in number of species a higher percentage of polyploids than diploids will survive.

Even if the theories claim that the increase of the frequency of polyploids is due to the greater hardiness or adaptability of polyploids than of diploids to more extreme climates, it is certainly not the only cause of the greater frequency of polyploids in more arctic regions. The results mentioned by LÖVE (1942) on the increase of osmotic pressure in the natural polyploid series of *Rumex* subg. *Acetosella* with an increase in polyploidy, and especially the results published by GYÖRFFY (1941) on the increased power of adaptability of the osmotic pressure to the climatic conditions as found within the autopolyploids studied by him, give a great support to the hypothesis that the greater hardiness is the most important property of the polyploids in the extreme climates. Another important physiological factor, however, is the photoperiodic reactivity of the plants. If the species is a short-day plant, it will never be able to live and flower in the long days of the arctic regions. According to ERNST (1941), autopolyploid material of *Antirrhinum majus* proved to be long-day plants, contrary to the diploid mother race, and LÖVE (1942) found that in *Rumex* subg. *Acetosella* the diploid species is to be regarded as a short-day plant while the polyploids are undoubtedly long-day plants. If a long-day reactivity or a fully neutral one to the photoperiod is assumed to be reatively more frequent in polyploids than in diploids, *the differences in hardiness connected with the differences in photoperiodism might be the primary causes of the greater frequency of polyploids than diploids at higher latitudes. When the climate becomes more arctic, only the most hardy long-day or photoperiodic neutral plants might survive.*

The present investigation can only show the general tendency in the geographical distribution of polyploids, as the regions studied are far too large to show any details. Closer analysis of the frequency of polyploids in edaphically unlike localities of a distinct area as made by ROHWEDER (1936) for the calcareous regions of Schleswig-Holstein, and HULDÉN (1941) for the flora in the different localities in Finland at which *Fraxinus exelsior* grows, is of course of much greater phyto-

geographical interest. A combined analysis of the phytogeographical and sociological details and the chromosome numbers of plants in localities with different edaphic and climatic conditions would undoubtedly give very interesting results on the real nature of the differences, especially if the frequencies of the polyploids were calculated on the basis of the phytogeographical data. When a number of such analyses have been made at different places in different countries, statistical comparisons of the real frequencies of polyploids on phytogeographically fully comparable localities at different latitudes will be of great interest to the workers in the field of cyto-ecology.

As mentioned above, the relatively high values of the frequency of polyploids on the Faeroes and Iceland are quite unexpected when the latitude and the mild climate of these Atlantic islands are compared with, e. g., the hardy climate in North Scandinavia. According to all the calculations in the present paper, these two countries show frequencies of polyploids between those obtained for Finland and Spitzbergen. This unexpectedly high frequency of polyploids is very possibly due to the isolation of these islands since the Tertiary Period. During the Ice Age a great number of insufficiently hardy plants died out and a relatively greater number of polyploids than diploids survived. The »over-wintering» hypothesis of elements of the Icelandic flora has already been brought forward by zoölogists (LINDROTH, 1931), geographers (THORARINSSON, 1937) and botanists (STEINDORSSON, 1937), and it is very much supported by the fact that a large number of the Icelandic plants show a northern or bipolar distribution in Scandinavia. Therefore, the present writers suggest that *the higher frequencies of polyploids on the Faeroes and Iceland than in, e. g., Finland and Norway are due to the higher percentages of »over-wintering» species on these islands and to a distinctly lower percentage of plant migration since the Ice Age.* It is not excluded that most of the Icelandic species which have migrated since the last Glacial Period have been brought there by Man during the last thousand years. The majority of these imported species might be diploids as found by TISCHLER (1935) in the adventitious flora of Schleswig-Holstein, which shows only about 30 per cent of polyploids.

Even if the statistical treatments of the data used for the present investigation give a clear view of the differences in polyploidy at different latitudes, it would be of very great interest if investigations on the frequency of polyploids were made in different regions of Asia and America with their higher number of species. An investigation of the frequencies of polyploids in different regions at the same latitude in

Eurasia would also be of great interest, as the number of species has also been shown to decrease from east to west (cf. E. V. WULFF, 1937). Generally, a closer co-operation between phytogeography and cytogenetics in different countries would be of the greatest value for the understanding of the distribution of the species in the world.

Acknowledgements. — The writers wish to thank Professor G. TISCHLER, Kiel, for his very kind help in giving them the recent figures of the flora of Schleswig-Holstein and Sicily, and Professor A. MÜNTZING, Lund, and Dr. O. TEDIN, Svalöf, for reading the manuscript and for valuable suggestions.

SUMMARY.

1. A close statistical investigation of the frequencies of polyploids in Timbuctoo, Sicily, Schleswig-Holstein, Denmark, Sweden, Norway, Finland, the Faeroes, Iceland and Spitzbergen has been made on the basis of the χ^2 analysis.

2. Studies of the frequencies within the whole floras show that a decided increase in polyploidy with an increase in latitude is statistically well founded, the general tendency of the increase always being the same from south to north.

3. Calculations of the two groups of Angiosperms show that polyploidy is more frequent within the monocotyledons than within the dicotyledons. Both the groups exhibit the same general tendency; the monocotyledons, however, show a decidedly higher rate of increase at higher latitudes.

4. The same general tendency to an increase in frequency of polyploids towards the north is also found when only the same families in the six Nordic countries and Spitzbergen are studied. The percentages of polyploid monocotyledons are somewhat lower than within the whole monocotyl floras, but within the dicotyledons the percentages are a little higher.

5. A statistical study of the numbers from the alpine regions of Pamir, Altai and Caucasus shows very significant differences between these regions in favour of the hypothesis of greater hardiness of polyploids than of diploids.

6. The halophytous flora of Schleswig-Holstein has not a significantly higher percentage of polyploids than the region as a whole. The halophytes of Roumania, however, show distinctly lower frequency of polyploids than the same flora of Schleswig-Holstein. This is assumed

to be due to the latitude as well as to the fact that the Roumanian coasts were never reached by the Ice Age.

7. The results of the calculations are briefly discussed. The statistical reduction of the material is found to give a very good support to the theory of a greater hardiness or adaptability of polyploids than of diploids in more severe climates.

8. The preponderance of species in tropical and moderate climates and the low number of species in cold regions are shown to be followed by an increase in the frequency of polyploids from temperate to cold regions. It is suggested that this fact is the result not only of the relatively greater hardiness or adaptability of polyploids than of diploids to more extreme climates but also of the possibly relatively higher frequency of long-day or neutral reactivity to the photoperiod within the polyploids than within the diploids.

9. The unexpectedly high values obtained for the Faeroes and Iceland may, it is suggested, be caused by a larger frequency of »overwintering» species and a lower percentage of plant migration since the last Glacial Period than are found in, e. g., Scandinavia. The possibility that almost all the migrated species have been imported by Man during the last thousand years is pointed out.

10. The desirability of detailed phytogeographical and sociological studies combined with analysis on the frequency of polyploids is stressed.

LITERATURE CITED.

1. Bonnier, G. and Tedin, O. 1940. Biologisk variationsanalys. — Stockholm.
2. Davidsson, I. 1937. Ny islenzk starartegund (*Carex irrigua* L.) og nokkrir fundarstadir fremur fagætra tegunda. — Natturufr. VII, pp. 30—32.
3. Dobzhansky, Th. 1937. Genetics and the origin of species. — New York.
4. Ernst, H. 1941. Die photoperiodische Reaktion bei autotetraploiden *Antirrhinum majus*. — Ber. Dtsch. Bot. Ges. 59, pp. 351—355.
5. Fisher, R. A. 1936. Statistical methods for research workers. 6th ed. — Edinburgh.
6. Flovik, K. 1940. Chromosome numbers and polyploidy within the flora of Spitzbergen. — Hereditas XXVI, pp. 430—440.
7. Györffy, B. 1941. The physiological and chemical conditions in polyploid plants. — Magyar Biol. Kut. Munk. 13, pp. 362—446.
8. Hagerup, O. 1930. Études des types biologiques de Raunkiaer dans la flore autour de Tombouctou. — Kgl. Danske Vidensk. Selsk. Biol. Medd. IX, 4, pp. 1—116.
9. — 1931. Über Polyploidie in Beziehung zu Klima, Ökologie und Phylogenie. — Hereditas XVI, pp. 19—40.

10. HULDÉN, E. 1941. Studien über *Fraxinus exelsior* L. — Acta Bot. Fenn. 28, pp. 1—250.
11. HYLANDER, N. 1941. Förteckning över Skandinaviens växter. I. Kärlväxter. 3 uppl. — Lund.
12. LINDROTH, C. H. 1931. Die Insektenfauna Islands und ihre Probleme. — Zool. Bidr. från Uppsala, Vol. 13, pp. 105—599.
13. LÖVE, A. 1941. *Ajuga pyramidalis* L. funnen på Island. — Bot. Not. 1941, pp. 235—236.
14. — 1942. Physiological differences within a natural polyploid series. — Hereditas XXVIII, pp. 504—506.
15. LÖVE, A. and LÖVE, D. 1942. Chromosome numbers of Scandinavian plant species. — Bot. Not. 1942, pp. 19—59.
16. MÜNTZING, A. 1936. The evolutionary significance of autopolyploidy. — Hereditas XXI, pp. 263—378.
17. OSTENFELD, C. H. and GRÖNTVED, J. 1934. The Flora of Iceland and the Faeroes. — Copenhagen.
18. ROHWEDER, H. 1936. Die Bedeutung der Polyploidie für die Anpassung der Angiospermen an die Kalkgebiete Schleswig-Holsteins. — Beih. Bot. Centralbl. 54, pp. 507—519.
19. SOKOLOVSKAJA, A. P. and STRELKOVA, O. S. 1938. Polyploidy in the high mountain regions of Pamir and Altai. — C. R. (Doklady) Acad. Sci. URSS, 21, pp. 68—71.
20. — and — 1940. Karyological investigations of the alpine flora on the main Caucasus range and the problem of geographical distribution of polyploids. — C. R. (Doklady) Acad. Sci. URSS, 29, pp. 415—418.
21. STEFANSSON, S. 1924. Flora Islands. 2. utgafa. — Kaupmannahöfn.
22. STEINDORSSON, S. 1934. Nyjar islenzkar plöntur. — Natturufr. IV, pp. 51—61.
23. — 1937. Jurtagrodurinn og jökultiminn. — Natturufr. VII, pp. 93—100.
24. TARNAVSCHI, I. T. 1938. Karyologische Untersuchungen an Halophyten aus Rumänien im Lichte zyto-ökologischer und zyto-geographischer Forschung. — Bul. Fac. Sti. Cernauti, 12, pp. 68—106.
25. THORARINSSON, S. 1937. Vatnajökull. Scientific results of the Swedish-Icelandic investigations 1936—1937. Chapter II. The main geological and topographical features of Iceland. — Geogr. Annaler 1937, pp. 161—175.
26. TISCHLER, G. 1935. Die Bedeutung der Polyploidie für die Verbreitung der Angiospermen. — Bot. Jahrb. 47, pp. 1—36.
27. — 1937. Die Halligenflora der Nordsee im Lichte cytologischer Forschung. — Cytologia, FUJII-Jub. Vol., pp. 162—170.
28. WULFF, E. V. 1937. Essay of dividing the world into phytogeographical regions according to the numerical distribution of species. — Bull. Appl. Bot., Genet. and Plant-Breeding, Ser. I, No. 2, pp. 315—368.
29. WULFF, H. D. 1937. Karyologische Untersuchungen an der Halophytenflora Schleswig-Holsteins. — Jahrb. wiss. Bot. 84, pp. 812—840.
30. ÖSTERGREN, G. 1942. Chromosome numbers in *Anthoxanthum*. — Hereditas XXVIII, pp. 242—243.

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QUANTITATIVE INVESTIGATIONS ON THE REACTION OF YEAST TO CERTAIN BIOLOGICALLY ACTIVE SUBSTANCES

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IT has recently been shown by BAUCH (1942 a and elsewhere) that yeast cells have a characteristic response to certain of those substances which are known to induce specific biological reactions. BAUCH studied the effect of c-mitotic and carcinogenic substances and of synthetic phytohormones. Although the primary reaction is different in different substances it is interesting to notice that representatives of all three kinds of substances bring about an increase in the cell-size of the yeast. This increase has been shown in certain cases to be constant through several re-inoculations, and, although the cytological behaviour of the cells has not been studied, BAUCH considered some of the giant races produced as tetraploid and even octoploid.

Among the tested substances camphor is outstanding in that it causes a primary effect which is so characteristic that it may be spoken of as a specific camphor reaction of the yeast. Under influence of camphor the cells behave in the following manner: »Sie sprossen nicht zu neue Hefezellen aus, sondern bilden einen Keimschlauch, der schliesslich zu einem kleinen, mehrzelligen Mycel auswächst» (BAUCH, 1942 a, p. 3). The decisive importance of the dosage is stressed: »Nimmt man zuviel Campher, so werden alle Hefezellen abgetötet, nimmt man zuwenig, so bleibt die Reaktion aus.» If cells which have been exposed to the camphor effect for some time are thereafter cultured under normal conditions, there are often found among them giant aberrants. Camphor in fact was found to be superior to the other substances in producing giant forms, at any rate so far as their frequency was concerned. Giant races produced by naphthalene acetic acid, on the other hand, were found to have a certain advantage from a practical viewpoint. They show a less pronounced retardation in their growth rate than is usually the case with the giant forms. BAUCH states, »dass ihre Wachstumsrate allem Anschein nach durchaus oder fast gleichwertig mit der der Ausgangsrasse ist» (1942 b, p. 421).

In the present paper we direct our attention to the significance of the dosage for the general poison effect and for the induction of the specific camphor reaction. We approach this problem with a quantitative method, using a procedure that has been worked out by LEVAN and ÖSTERGREN (unpublished) for the treatment of higher plants with known concentrations of c-mitotic substances. As these substances with a few exceptions are very difficult to dissolve in water, we use absolute ethyl alcohol as a solvent. Small quantities of the alcoholic solution are mixed in a large excess of water, or as in the present case of yeast cultures of wort. When needed, a quantity of

TABLE 1. *The increase in cell number in pure wort.*

Series	Original cell number per mm^3	Cell number after 20 hours	Relation to the original cell number	Cell number after 48 hours	Relation to the original cell number
Colchicine	280	16125	57,6	47000	167,9
Acenaphthene	280	7870	28,1	31400	112,1
α-chloronaphthalene	630	10140	16,1	—	—
β-chloronaphthalene	630	12950	20,6	—	—
Naphthalene acetic acid	850	9925	11,7	30750	36,2
Camphor.....	440	7960	18,1	22800	51,8
Camphor, new series.....	570	11975	21,0	30725	53,9
Borneol	370	7130	19,3	27350	73,9
Borneol, new series	1100	14725	13,4	—	—

pure alcohol is also added in order to get the same alcoholic concentration in all solutions.

The following substances were tested: acenaphthene, α- and β-monochloronaphthalene, α-naphthalene acetic acid, camphor, and borneol. A wide range of concentrations was used: from 3×10^{-7} mol to saturated solution, i. e. to solutions where a permanent precipitation originated, when the alcoholic solution was mixed with the wort. All solutions, including the controls, contained 1 % alcohol. The colchicine series was prepared directly from an 8 % colchicine solution in water.

The colchicine series was kept in 10 cc FREUDENREICH flasks, in the other series ordinary soda water bottles were used with 100 cc culture fluid in each. At the start of the experiment there was added in the colchicine series $^1/_{10}$ cc and in the other series 1 cc of a yeast culture which was in lively fermentation. The original frequency of

yeast cells was determined in each series, and the usual concentration was found to be some hundred cells per mm^3 (see Table 1). The bottles were kept in a thermostat at 25° C and the number of cells was counted after 20 hours and in certain cases also after 48. The countings were made in BÜRKER's counting chamber. During the estimation all bottles of the series concerned were kept at 0°, in order to prevent changes in cell number from occurring during the counting. Of each concentration $^1/_{10}$ mm^3 was counted after 20 hours and $^1/_{25}$ mm^3 after 48.

Since certain of the substances, as, for instance, camphor, are highly volatile, the concentration data of the first counting must be considered more reliable than those of later countings. When the culture bottle has been shaken once and the carbon dioxide has been let out, the concentration of the tested substance in the wort must have decreased considerably.

All experiments were made with the same clone of a yeast, which has been under constant biological control during 50 years by the Stockholm Brewery Co., and which is used in their beer manufacture. Its properties and reactions under different conditions are consequently very well known.

I. THE GENERAL POISON EFFECT OF THE DIFFERENT SUBSTANCES.

The general poison effect brought about by the different substances was determined partly as the arrest in augmentation of the cell number caused by the substance, partly as the frequency of dead cells per unit volume. An idea of the normal growth of the yeast in pure wort during the experiments can be obtained from Table 1, which shows the number of cells per mm^3 in the controls of the different series. If the colchicine series is excepted, which was treated somewhat differently from the other series, broadly speaking the following behaviour is encountered: during the first 20 hours the cell number is augmented from some hundred cells to about 10000 cells per mm^3. After the next 24 hours the number is about 30000 cells per mm^3. The rather considerable differences present between the different controls are no doubt due to slight differences in the treatment. We tried to make the treatment *within* each series as uniform as possible; *between* the different series certain differences occurred owing to variations in the original cell concentration and to the presence of different numbers of cultures in different series, which necessarily caused differences in the time spent

on the counting and consequently also introduced a variation in the time of cooling the cultures, and so on.

In Table 2 a survey is given of the treatments made and of the main results obtained. As seen from this table, the concentrations are varied with intervals of 10 times: 0,1, 0,01, 0,001, etc. Between each such interval two concentrations are inserted corresponding to $^1/_3$ and $^2/_3$ of the size of the interval (in the borneol series, however, each interval is divided into only two parts). The saturated solutions (*italics* in each first row of the different substances in the table) are present at the following intervals:

colchicine:	0,1	mol =	3,9932 %
acenaphthene:	0,0001	» =	0,0015 »
α-chloronaphthalene:	0,0003	» =	0,0049 »
β-chloronaphthalene:	0,0001	» =	0,0016 »
naphthalene acetic acid:	0,003	» =	0,0558 »
camphor:	0,003	» =	0,0507 »
borneol:	0,003	» =	0,0509 »

Table 2 shows the augmentation in cell number under influence of the different substances during 20 and 48 hours respectively. In order to render the different substances as far as possible comparable with each other, the increase in cell number after 20 hours in pure wort is used as a unit and made equal to 100. All absolute values may be calculated, however, with use of Table 1. In Table 2 there is also given for each substance the percentage of dead cells.

From Table 2 it is immediately seen that the effect of the different substances is greatly varying. Colchicine and acenaphthene have no perceptible effect. Even in saturated solutions the cell divisions take place unimpeded. In the colchicine series all values are gathered around 100, neither a noxious influence, nor a stimulation, as observed by RICHARDS (1938), could be demonstrated. In the case of acenaphthene all values lie below 100, probably owing to the control incidentally having received too high a number. Anyhow, it is probably not a question of an arrest of the cell divisions, because this arrest would then be greater in the high concentrations than in the weak. The lethality, too, shows about the same values all through the colchicine and the acenaphthene series.

No differences in the process of fermentation could be found between the treated cultures and the controls either in the colchicine or

TABLE 2. *The increase in cell number, the lethality and the occurrence of camphor forms.*

Molar concentration			10^{-1}	7×10^{-2}	3×10^{-2}	10^{-2}	7×10^{-3}	3×10^{-3}	10^{-3}	7×10^{-4}	3×10^{-4}	10^{-4}	7×10^{-5}	3×10^{-5}	10^{-5}	7×10^{-6}	3×10^{-6}	10^{-6}	7×10^{-7}	3×10^{-7}	Pure wort
Colchicine	Day 1	A [1]	*107*	86	93	98	95	103	105	110	96	111	105	81	90	105	95	90	—	—	100
		L [1]	2,0	2,9	2,7	2,4	1,0	0,8	1,8	1,4	1,9	1,0	2,5	1,3	2,9	2,5	3,9	2,7	—	—	1,7
	Day 2	A	406	246	—	—	—	—	—	285	—	—	—	—	—	—	—	—	—	—	295
		L	3,1	7,7	—	—	—	—	—	6,6	—	—	—	—	—	—	—	—	—	—	7,7
Acenaphthene	Day 1	A	—	—	—	—	—	—	*80*	—	*96*	*83*	91	96	95	85	85	—	—	—	100
		L	—	—	—	—	—	—	2,8	—	2,8	5,1	2,9	3,2	3,7	2,5	1,9	—	—	—	4,3
	Day 2	A	—	—	—	—	—	—	550	—	—	—	386	—	—	—	—	—	—	—	410
		L	—	—	—	—	—	—	2,9	—	—	—	4,4	—	—	—	—	—	—	—	2,9
Chloronaphthalene α	Day 1	A	—	—	—	—	—	*0*	*0*	*0*	*0*	43	60	79	—	104	—	—	—	—	100
		L	—	—	—	—	—	100,0	100,0	98,6	95,8	9,4	1,4	2,2	—	0,6	—	—	—	—	1,5
Chloronaphthalene β	Day 1	A	—	—	—	—	—	*7*	*23*	*19*	*20*	*76*	62	76	—	94	—	—	—	—	100
		L	—	—	—	—	—	15,8	6,6	5,9	4,2	2,2	3,1	1,4	—	1,4	—	—	—	—	1,4
Naphthaleneacetic acid	Day 1	A	—	—	—	*0*	*0*	*4*	29	99	98	135	107	111	112	126	100	107	120	101	100
		L	—	—	—	98,2	96,3	6,4	2,2	2,0	1,5	1,5	1,0	1,6	1,1	2,6	1,3	2,8	1,9	1,3	1,8
		C [1]	—	—	—	0	0	3	4	4	4	0	3	0	2	1	2	0	0	1	0
	Day 2	A	—	—	—	0	0	35	146	271	298	—	348	—	243	—	257	—	328	—	330
		L	—	—	—	99,5	92,2	3,7	2,0	3,0	1,6	—	2,0	—	3,5	—	3,8	—	2,3	—	2,6
Camphor	Day 1	A	—	—	—	*0*	*0*	*43*	79	79	92	95	86	83	88	89	86	78	—	—	100
		L	—	—	—	81,8	27,7	6,6	3,3	3,7	3,8	5,1	2,3	3,5	4,0	2,4	4,1	7,3	—	—	2,1
		C	—	—	—	0	250	220	20	← Single camphor forms →									—	—	0
	Day 2	A	—	—	—	0	46	228	292	303	—	299	—	—	249	—	—	342	—	—	297
		L	—	—	—	100,0	7,8	3,6	2,8	3,3	—	2,2	—	—	3,9	—	—	1,8	—	—	2,7
		C	—	—	—	0	230	80	20	← Single camphor forms →									—	—	0
Borneol	Day 1	A	—	—	—	*0*	*0*	*20*	58	90		112	100		103	103		102	—	—	100
		L	—	—	—	90,0	63,3	5,2	0,7	1,4		2,4	0,7		2,5	0,4		1,0	—	—	0,6
		C	—	—	—	0	0	20	20	0		0	0		0	0		0	—	—	0
	Day 2	A	—	—	—	0	0	—	366	—		—	—		—	—		—	—	—	399
		L	—	—	—	100,0	73,1	—	1,3	—		—	—		—	—		—	—	—	2,5
		C	—	—	—	0	5	12	5	—		—	—		—	—		—	—	—	0

[1] A = Augmentation in cell number. — L = % lethality. — C = Number of camphor forms per mm³.

in the acenaphthene series. The treated cultures finished their fermentation as rapidly as the controls, the foam head and the sediment layer had the same thickness and the same appearance as in the controls. During a fortnight microscopical samples were taken daily from the different concentrations. In the two strongest concentrations of colchicine we now and then came across camphor forms, although never more than bicellular forms. Since such types may also occur in normal untreated yeast, we do not venture to ascribe their presence to the action of the colchicine. But no doubt their frequency (1 or 2 per mm^3) was greater than normally. In the acenaphthene series no camphor forms were met with. Nor did we find any strikingly great occurrence of giant cells. According to BAUCH the temperature is of importance for the action of acenaphthene, its polyploidogenic effect increasing with the temperature. One bottle of the strongest acenaphthene concentration was kept at 30° during the fermentation and for one week after its end. However, we found no deviating cell types in this culture either. Thus, it may be concluded that neither colchicine nor acenaphthene, which both have a very pronounced c-mitotic effect in higher plants, have any such effect in yeast, at any rate not by inducing c-mitoses. Nor have they any clearly poisonous action.

All the other tested substances behaved in another way. α-chloronaphthalene arrests the cell divisions completely in the four strongest solutions, which were all saturated but had different excesses of the substance. An arresting effect on the cell multiplication may be noticed all the way down to a 3×10^{-5} molar solution. The four first concentrations evidently have a strong poisonous action, the lethality lying between 96 and 100 %. The β-form of chloronaphthalene behaves in a similar manner, though a marked difference in its poisonous effect may be seen, this being much weaker. Even in the strongest concentration of β-chloronaphthalene the cell growth has not stopped altogether, and the lethality is 16 % as against the total lethality of the α-form in this concentration. No camphor forms were seen in the chloronaphthalenes, but in the strongest solutions the cells often took a more spherical shape than usual, and sometimes cells with an amoeboid appearance were seen.

Naphthalene acetic acid is also poisonous in the strongest concentrations. The cell multiplication was checked completely in the first two concentrations and partly in the two following ones. The lethality approaches 100 % in the two strongest solutions. During the first day the weak solutions of naphthalene acetic acid very strikingly showed

TABLE 2. *The increase in cell number, the lethality and the occurrence of camphor forms.*

a greater cell number than the control. This is the case in the range $10^{-4} - 3 \times 10^{-7}$ mol (= 18—0,006 parts per million), and it indicates a stimulation of the cell divisions. This condition is perhaps not so surprising, considering the effects of this substance on higher plants. The numbers from the second day do not, however, confirm the observation of a stimulation. Further experiments are therefore required before a decision on this point can be reached. In the concentrations 3×10^{-3}—7×10^{-5} there were found at the first counting single camphor forms (3—4 per mm^3), and also in lower concentrations camphor forms were met with now and then. Even if most of them consisted of only two cells, and might also be noticed at times in untreated yeast, there also occurred camphor forms with as many as seven cells, and such forms are certainly never present normally. Their origin must therefore be ascribed to the treatment. It would thus seem that the camphor reaction is not absolutely specific to camphor and that other substances may also have this effect. On examining the cultures after 48 hours no regular occurrence of camphor forms could be found. This indicates that the reaction in this case constitutes the effect of a shock, and that the cells may gradually become insusceptible to the substance.

Both camphor and borneol have a considerable poison effect in strong concentrations. The cell propagation stops and the lethality is 80—90 %. After two days in the strongest solution all cells have the appearance of being dead. Some of them, however, may keep alive even through a still longer treatment. If transplanted into new wort seemingly dead cells may start fermentation even if they have undergone treatment for 6 days in a culture medium containing 0,01 mol camphor. The starting of the fermentation is very much retarded, however, and full fermentation may be several days later than normally.

The strongest solutions of the camphor series show no fermentation at all during the first few days. But gradually fermentation begins, and long after the fermentation in the control and the weaker solutions is finished, the more concentrated solutions show a lively fermentation. Their camphor concentrations have evidently decreased, partly at the opening of the bottles for sampling, partly, maybe, as a result of the vital processes of the yeast cells. It appears, then, that a sufficient number of cells always succeed in keeping alive for the starting of the fermentation as soon as the camphor concentration is no longer noxious.

Camphor forms are found both in camphor and borneol. They are formed most frequently in the strongest solutions in which cellular

growth may occur at all. It is seen from Table 2 that most camphor forms are present in the camphor series in the solutions 0,007—0,003 mol, while single camphor forms occur in all concentrations, even the weakest. The active zone in borneol is 0,003—0,001 mol. Lower concentrations contained no camphor forms in this case. The frequency of camphor forms was very much less in borneol than in camphor.

II. A DETAILED EXAMINATION OF THE INFLUENCE OF CAMPHOR AND BORNEOL.

We learn from the preceding that in those substances which have any effect the critical concentrations are situated in that zone where a precipitation of the substance is present, i. e. in saturated concentrations. A characteristic feature, also observed in higher plants by LEVAN and ÖSTERGREN (unpublished), is that even within the saturated range the different solutions give different results. The greater the undissolved excess of the substance is, the more extreme is the poison effect. It is evident that the quantity of undissolved substance present has an effect on the biological reaction.

For various reasons we decided to make a detailed scrutiny of the critical concentration range in camphor and borneol. Our object was to study the dependence of the peculiar camphor reaction on the concentration, and we considered it worth while to compare the effect of two so closely related substances as camphor and borneol, which differ only in one radical, $>C{:}O$ in one case, $>CHOH$ in the other. We therefore prepared a series of solutions which held equal thousandths between 0,01 and 0,001 mol of camphor, and half thousandths between 0,006 and 0,002 mol of borneol. We determined as closely as possible the maximal solubility in wort of the two substances, and it was found that the saturation point of camphor is situated between 0,004 and 0,005 mol, and that of borneol between 0,001 and 0,002 mol. The solubility in water of these two substances has been determined by RHODE (1922) as 0,0112 and 0,0042 mol respectively. These values are about three times higher than our figures, but the relation between the solubility of camphor and borneol is about the same. It should be noticed that the camphor used by RHODE and us is the synthetic and optically inactive form.

Table 3 gives a survey of our results. Concerning cell propagation and lethality the figures in this table agree rather well with the more preliminary values of Table 2. In these latter experiments, however,

a new feature is observed, which was not clearly seen from Table 2: there is a decided difference in the poison effect between camphor and borneol, the former substance being considerably less poisonous than the latter. Borneol checks any cell propagation all the way down to 0,0035 mol, while an augmentation of the cell number may be noticed in 0,006 mol camphor. Likewise the lethality is greater in borneol than in camphor if identical concentrations are compared.

The most interesting result of these series is the behaviour of the camphor forms. Thus, we find that in the camphor series their percental occurrence increases with increasing quantity of substance up to 0,007 mol, where almost all living cells are camphor forms. In this solution no increase in cell number has taken place, which means that all cells which have divided have grown out into camphor forms.

TABLE 3. *Examination of the critical zone of camphor and borneol.*

Concentration in 10^{-4} mol			100	90	80	70	60	55	50	45	40	35	30	25	20	10	0
Camphor	Day 1	A [1]	0	0	0	0	0,2	—	9	—	30	—	50	—	60	66	100
		L [1]	97,9	96,3	94,9	38,2	32,2	—	22,2	—	2,3	—	4,7	—	2,5	5,3	2,7
		C [1]	0	0	0	91,5	85,0	—	34,1	—	5,1	—	1,5	—	0,7	0,3	0
Borneol	Day 1	A	—	—	—	—	0	0	0	0	0	0	20	25	51	—	100
		L	—	—	—	—	97,1	84,7	82,8	78,2	40,3	17,5	5,2	4,9	4,3	—	2,7
		C	—	—	—	—	0	0	0	33,3	22,5	21,2	6,6	1,9	0,5	—	0

At the recording each camphor form was always counted as one cell, even if it was built up of many cells, the object of the cell count being not to show the number of mitoses which had occurred but the number of free organisms. For practical reasons it was found impossible to decide exactly at what point in the budding process the mitosis had finished. Therefore budding cells were counted as one, until the bud cell had separated from the mother cell. But during the development of the camphor forms the cell-division products do not separate but form colonies.

In borneol we find the same behaviour as in camphor: the frequency of the camphor forms increases with growing concentration up to 0,0045 mol, which is the highest concentration permitting any cell growth. In borneol the top point of the curve never reaches higher than to 33 % of the living cells. Speculations may be indulged in as to whether a

[1] A = Augmentation in cell number. — L = Lethality %. — C = Camphor forms in percentage of living cells.

greater excess of borneol would have effected an increase to 100 % of the frequency of camphor forms if the poison effect of borneol had been weaker and had not altogether checked the cell growth.

III. THE DEVELOPMENT OF THE CAMPHOR FORMS.

The detailed course of the development of the camphor forms will be described elsewhere. On this occasion only the coarse outlines of this interesting process will be touched upon. Under influence of the critical concentrations 0,006—0,007 mol camphor or 0,0035—0,0045 mol borneol the budding process of the yeast cells does not occur normally. Instead, the cells stretch in length considerably. They become oblong and are often swollen at one end (Fig. 1 *b*). Now this cell may be divided at its middle by a cell wall, in the same way as fungous hyphae divide, or a bud may originate at the thick pollen tube-like end of the cell. This bud grows out into a new tube-like formation which is separated from the mother cell by a cell wall. In both cases the two resulting cells will hang together. The further cell growth occurs rather regularly (Fig. 1 *c*—*g*). Often one to three new cells are developed at each end of the initial cell, and these new cells grow by developing one to three cells at their distal end. Thus, the mycelium will grow radially in all directions. Since several cells are often developed by each of the cells of the preceding cell generation, there is gradually formed a thick texture of hyphae, recalling the structure of the apothecia of higher fungi.

We made some records of the number of cells in the colonies grown for 20 and 48 hours in the different camphor solutions. After 20 hours we found on an average 3,8—5,0 cells per colony in the different cultures. The maximum was 12—13 cells. The average number of cells per colony was also similar after 48 hours: 2,8—4,6. The small decrease in the value of the means may be caused by many new cells having started their development into camphor forms. The maximum of cells per colony after 48 hours was about 30. It is extremely typical that a solution which the first day contained 100 % camphor forms had already on the second day only about 30 % camphor forms. On the third and fourth day the same solution had preponderantly normal cells, and it could be seen that typical camphor colonies began to produce in their exterior layers normally shaped cells, which consequently loosened from the colony (Fig. 1 *i*).

In solutions the great camphor excess of which had at first made

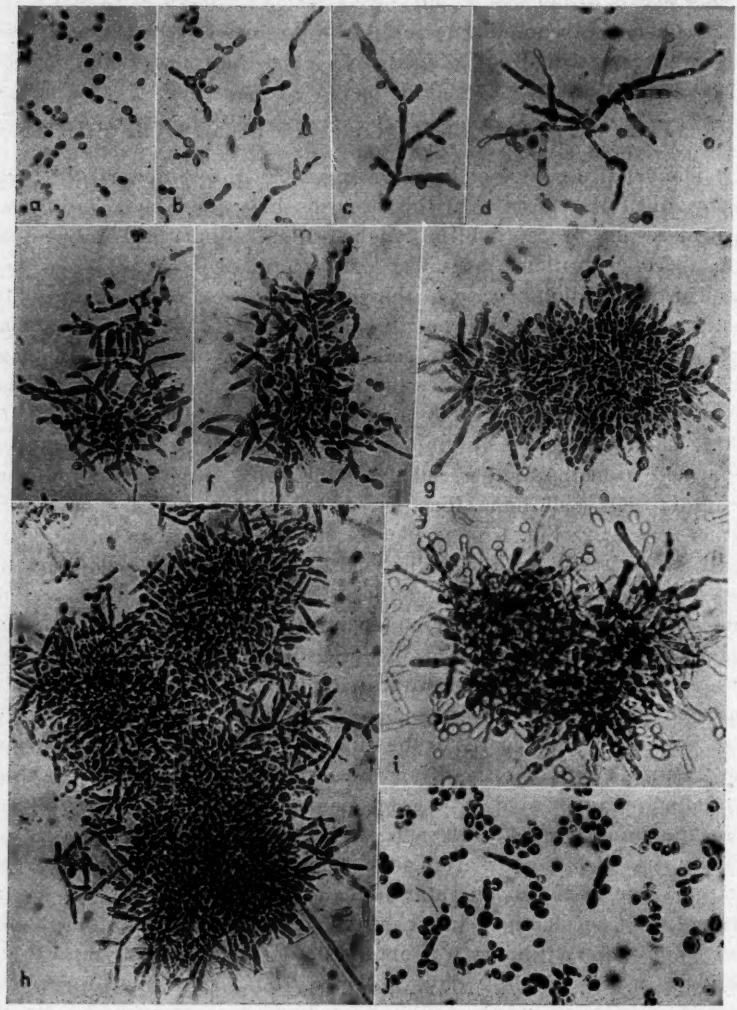

Fig. 1. *a*: Untreated, normal yeast cells, *b*—*h*: the development of camphor forms, *b*: after 20 hours, *c*—*d*: 48 hours, *e*—*g*: 3 days, *h*: 8 days, *i*: one camphor colony, which produces normal yeast cells, after the concentration of camphor has decreased below the threshold value of the camphor reaction, *j*: a sample of untreated, aging yeast; note the occurrence of »camphor forms» and of giant cells. — × 200.

impossible any growth of the cells as, for instance, 0,01 mol, there started on the fourth or fifth day a development of camphor colonies. Since these cultures showed very slight fermentation, their camphor concentration could be held more constant than was possible in the fermenting cultures, and in consequence very large colonies could develop. When the experiment was discontinued on the tenth day, camphor colonies with thousands of cells were not infrequently observed (Fig. 1 *h*). It can be said that *Saccharomyces cerevisiae* had changed into a colony-forming organism. Other experiments are in progress to investigate how far these colonies can be brought to grow. In view of the fact that yeast is probably a secondarily primitive organism, which must be derived from some group of the higher fungi, it is of some interest to study the morphology of these artificially produced thallus-like bodies. Perhaps some clue to the relationships of yeast may be discovered by such studies.

IV. DISCUSSION.

The present results make it obvious that there is no reason whatever to draw a parallel between the action exerted on yeast by any of the tested chemicals and the colchicine effect on higher plants. Colchicine induces c-mitoses on higher plants. The gist of this process is that the chromosomes divide but the cells remain undivided. It has been fully shown that none of the tested substances in their active concentrations prevent cell divisions in yeast. The behaviour of the chromosomes has so far not been studied in detail, but by suitable cytological methods one of us has shown that the yeast nuclei divide and separate even after a long treatment with camphor, monochloronaphthalene, or acenaphthene (LEVAN, unpublished).

As mentioned earlier, BAUCH (l. c.) obtained numerous giant »mutations» in his treated yeast material. Although our experiments along this line are not yet concluded, we may mention that in our material we have not so far obtained any morphological deviations which we dare to interpret as an induction of tetraploidy or octoploidy. It is true that very often giant cells occur, but such cells are found also quite spontaneously, especially in aging yeast. Fig. 1 *j* shows a sample of an old yeast culture, left for 5 weeks after the end of the fermentation. It contains a high percentage of giant cells, some of which are growing out into typical »camphor forms». These camphor forms are usually built up from two cells, one spherical and one tube-like cell,

but in a few instances camphor forms with two or even three tube-like cells were observed. We made several one-cell-cultures of such giant cells, and we found that they were usually non-viable. But in those cases where they could be brought into fermentation they had always reverted into normal size and appearance. The same was the case with those plate cultures which were made from the different treated materials, including five plates of the camphor culture with more than 90 % camphor forms. In no case did we obtain any giant colonies with a deviating macroscopical appearance. The microscopical examination of some 100 colonies showed the cells to be of a normal size and shape. However, our attempts to obtain constant giant forms will be continued.

The c-mitotic substances have also another effect on higher plants, an effect which is to a certain degree autonomous from the c-mitotic effect (LEVAN, 1942), viz. they bring about an increase in the cell volume (the c-tumour reaction). This effect is of the same nature as the swellings caused by phytohormones. Camphor and borneol no doubt have such an influence on yeast. The camphor forms have cells which are several times larger than normal yeast cells. It is known from the higher plants that this swelling, especially when it acts on older non-meristematic cells, brings about an augmentation of the chromosome number of the cells in question. While the c-mitotic substances act on the most active mitotic stages and prevent the anaphase movement, the phytohormones act on resting nuclei and the chromosome doubling takes place within the nuclear membrane through endomitosis. Since BAUCH, in the progenies of camphor-treated yeast cells, produced races with larger cell volume than the original strain, it does not seem to be improbable that a doubling of the chromosomes has occurred rather by the endomitotic mechanism than by c-mitosis. It should be added, however, that camphor is in any case really a c-mitotic substance. In *Allium* LEVAN and ÖSTERGREN obtained in the strongest solution used (0,05 % camphor) the typical c-mitotic response. This c-mitotic effect was not far away from the lethality limit of the *Allium* cells.

It is of interest to compare the poison effect on yeast of the different substances with their poison effect on higher plants. Root cells of *Allium* are killed by a 0,004 % solution of α-chloronaphthalene, if it is allowed to act for 24 hours, or by a 0,002 % solution acting for 48 hours. Yeast shows more than 95 % lethality after being under the influence of solutions above 0,005 % α-chloronaphthalene for 20 hours. Thus, the lethality limit for this substance is of the same order of magnitude in *Allium* and yeast. Solutions of naphthalene acetic acid above 0,005 %

cause lethality in *Allium*. Yeast, on the other hand, endures solutions more than 10 times stronger. First in concentrations of 0,1—0,2 % is yeast killed in a higher proportion than 95 %. Camphor solutions of 0,05 % kill *Allium* roots in 3 days (LEVAN and ÖSTERGREN), while the lethality of yeast is located considerably higher: the influence of 0,15 % camphor during 2 days brings about a total lethality, while the poison effect of a 0,10 % solution is only slight.

The specific camphor reaction in yeast, which has earlier been ascribed only to camphor, may also be caused by borneol and to a less degree by naphthalene acetic acid. BAUCH (1942 a) made qualitative tests of a great number of camphor derivatives: camphoric acid, camphoric acid sodium, camphoric anhydride, camphoric acid imide, monobromated camphor, camphoric carbonic acid, camphoric sulphonic acid, camphoric sulphonic acid sodium, and camphoric oxime. None of these substances gave the reaction. It must be pointed out, however, that neither did borneol in a qualitative test made according to BAUCH, i. e. with hanging droplet cultures above small pieces of borneol. It therefore cannot be excluded that one or more of the above substances may give the camphor reaction if tested according to our procedure.

From the quantitative analysis it is seen how narrow the concentration zone in reality is in which the typical camphor reaction is obtained to any considerable degree. This zone is 0,006—0,007 mol for camphor and 0,0035—0,0045 mol for borneol. In view of the general lethality attending concentrations immediately above these it is not surprising that a total camphor response is only obtained with great difficulty by qualitative methods. BAUCH states that in no case all cells of the droplet cultures show the camphor reaction. This is probably due to the fact that even those small differences in concentration which must be present within the droplets and between different droplets of the same drop culture are sufficient to make the reaction unequal. In fact, this camphor reaction of yeast cells is so sensitive that it could be used as a biological method for an exact determination of the concentration of camphor.

It is remarkable that both in camphor and borneol the full reaction is obtained immediately above the saturation point of solubility. For a maximal reaction to be obtained, the presence of a certain excess of solid substance is evidently necessary. The quantity of this solid excess is of decisive importance. As the solution must be considered saturated in this concentration range, the reaction must be assumed to depend on some digestive process in the yeast cell by which camphor is de-

composed. The deficit thereby arising may evidently be filled easier if a greater quantity of solid camphor is at hand. The biological decomposition of camphor may also explain the gradual lowering of the camphor concentration even in culture bottles which do not ferment and which have not been opened.

SUMMARY.

An investigation is made of the reaction of yeast to known concentrations (10^{-1}—3×10^{-7} mol solution) of colchicine, acenaphthene, α- and β-monochloronaphthalene, α-naphthalene acetic acid, camphor, and borneol. Colchicine and acenaphthene, even in strong concentrations, had no effect on the cell propagation and the lethality. Saturated concentrations of the other substances had a varying poison effect. The conditions of the specific camphor reaction are determined. This reaction is induced by camphor and borneol, and to small extent also by naphthalene acetic acid. The most active concentrations for the induction of camphor forms are 0,006—0,007 mol camphor and 0,0035—0,0045 mol borneol. Some particulars concerning the development and morphology of the camphor forms are given. This camphor reaction of yeast is discussed in relation to the c-mitosis and the c-tumour reaction of higher plants.

LITERATURE CITED.

1. BAUCH, R. 1942 a. Experimentelle Mutationsauslösung bei der Hefe durch chemische Stoffe. — Wochenschrift für Brauerei.
2. — 1942 b. Über Beziehungen zwischen polyploidisierenden, carcinogenen und phytohormonalen Substanzen. Auslösung von Gigas-Mutationen der Hefe durch pflanzliche Wuchsstoffe. — Naturwissensch. 30: 420—421.
3. LEVAN, A. 1942. The macroscopic colchicine effect — a hormonic action? — Hereditas XXVIII: 244—245.
4. RHODE, H. 1922. Löslichkeit, Capillaraktivität und hämolytische Wirksamkeit bei Terpenderivaten. — Biochem. Zeitschr. 130: 481—496.
5. RICHARDS, O. W. 1938. Colchicine stimulation of yeast fails to reveal mitosis — Journ. of Bacteriol. 36: 187—195.

MEIOSIS IN A NULLISOMIC AND IN AN ASYNDETIC GODETIA WHITNEYI

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CYTOLOGICAL studies of wild-growing races of *Godetia Whitneyi*, from which seeds had been collected and plants cultivated by Dr. GUNNAR HIORTH in Ås, Norway, led to the discovery of monosomic (2n—1) plants in some races (HÅKANSSON, 1942). These plants had 13 chromosomes; during meiosis a univalent appeared that never showed any tendency to pairing with any other chromosome. Four cases of the monosomic condition were found, namely in the races Santa Rosa 6, Horse Mt., Briceland, and Tocaloma (respecting these races see HIORTH, 1942). In the two first-mentioned cases monosomics were found among plants obtained from seed, and they must be very common in the localities from which the seed was collected. The other monosomics were discovered in another way. A study was being made of the pattern of certain markings on the cotyledons, Briceland-C^{st} and Tocaloma-C^{pa} (the latter formerly called C-Tocaloma). On being crossed with a race without markings, Bremen, they exhibited a peculiar mode of inheritance, and plants with markings, on being examined, were found to be monosomic. Genetical experiments yielded C^{st} and C^{pa} respectively in great excess. According to examination of root-tips from germinating seed, C^{st} seems to be almost constantly monosomic.

It is rather surprising that monosomics should be so easily formed in *Godetia Whitneyi*, as the haploid chromosome number of this species is only 7. Different numbers occur within the genus, but the lowest is 7, and this seems to be the lowest and commonest within the whole family of Onagraceae and appears to be a definite basic number. Certainly monosomics are known from species with high chromosome numbers, there being, for instance, several in *Triticum vulgare*, *Avena sativa*, *Nicotiana tabacum* and *rustica*, but they are rare among the lower numbers. Among diploid grasses such as rye, barley, and maize they are unknown. Nor are they known in *Oenothera* or *Datura*, although 2n—1 branches have been observed on diploid *Datura* plants. GOODSPEED and AVERY (1929) found, however, a monosomic in *Nicotiana alata* ($n = 9$), and CLAUSEN (1930) may also be cited as to the condition

in *Viola Orphanidis*. In progenies from monosomics there should theoretically occur so-called nullisomics (2n—2) which have lost both the homologues. SEARS (1941) obtained seven nullisomics from *Triticum vulgare*, but from *Nicotiana* they are not known, and LAMMERTS (1932) failed to get any from *N. rustica* (n = 24) in spite of specially arranged experiments. As favourable conditions for the formation of nullisomics appeared to exist in *Godetia Whitneyi*, a watch was kept for such. From monosomics of Horse Mt. and Santa Rosa 6 HIORTH also obtained very sterile plants that he took to be nullisomic, but only those from Santa Rosa 6 turned out to be such.

SANTA ROSA 6 ff × BREMEN, F_2.

This cross gave rise to nullisomics in F_2. *ff* indicates absence of petal spot; Bremen has spot. F_1 contained pollen-fertile and partially pollen-sterile plants, all with petal spot, but the latter were undoubtedly monosomics. A fertile F_1 plant gave normal, large-flowered *ff* plants. Two partially pollen-sterile F_1 plants produced *ff* plants with small flowers, greatly reduced anthers, and many sterile pollen-grains. Among the well developed grains were many with four lobes instead of three. In spite of their numerous peculiarities these plants can in no way be denoted as stunted. They are, however, quite sterile, no seed formation having resulted after numerous crosses. They are produced in great numbers, more than 25 per cent. Of the plants with petal spot the great majority were partially pollen-sterile. Some particulars regarding the cross have been given to me by HIORTH.

Four *ff* plants with small flowers (from S 771—1942) were examined and all were found to be nullisomics. They ought to be described as *00* rather than *ff*, and the partially pollen-sterile as *F0*. They had 12 chromosomes at somatic divisions in petals, and meiosis could be studied in all. No disturbances in the development of the anthers took place, the pollen-chambers were full of PMCs, which passed through a perfectly normal development. Complete asyndesis prevails with 12 I. Only very seldom, in less than $^1/_2$ % of the PMCs, was there a II observed. Meiosis is very similar to that of the haploid *G. Whitneyi* and the asyndetic hybrid *G. Whitneyi* × *deflexa* (HÅKANSSON, 1940 b, 1941), and its course therefore needs only a very brief description.

The univalents are at first tolerably spread in the PMCs, but as a rule tend to form an equatorial plate (Fig. 1 *a*), although this proceeds

slower than in diploid forms, and the plate is often incomplete. Frequently, however, all the univalents are seen in the plate (Fig. 1 *c*). No division of the centromeres takes place at this stage. On the other hand, there is often distinctly seen a movement of undivided univalents towards the poles of the spindle, just as in the previously-described

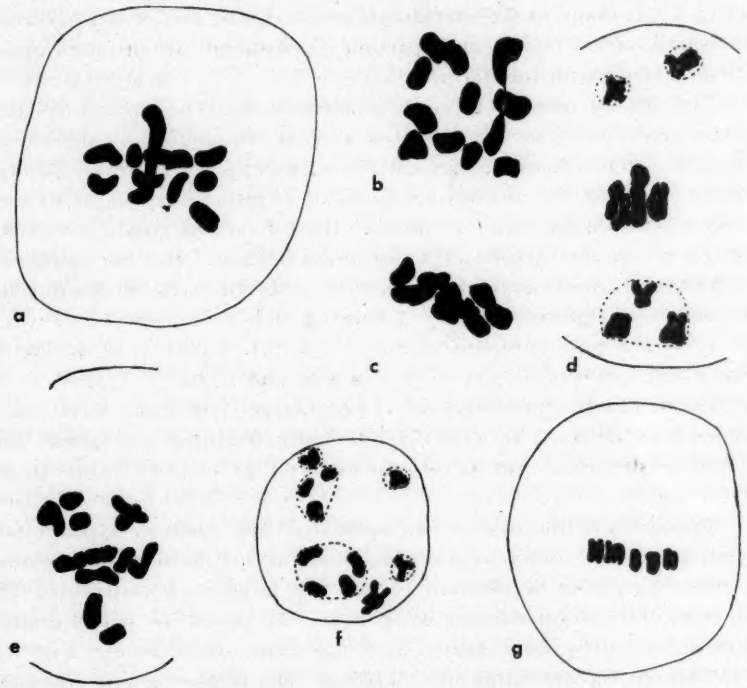

Fig. 1. A nullisomic from Santa Rosa 6 × Bremen, F_2. — *a*: 1 II + 10 I. — *b*: 1 II + 10 I. — *c*: the univalents have formed an equatorial plate. — *d*: telophase 1, some chromosomes lag. — *e*: anaphase 1, some univalents move to the poles. — *f*: interkinesis. — *g*: division of a restitution nucleus. — × 2250, *f* × 875.

forms. An anaphase movement of this kind is mostly not performed by all the chromosomes. Occasionally there is no such movement and a restitution nucleus is formed. This nucleus, however, is formed considerably less often than in the haploid, where it is extremely common, but more frequently than in the asyndetic hybrid. There are often formed two pole groups that give rise to two interkinesis nuclei. Most commonly, however, the chromosomes are much spread when inter-

kinesis sets in, with the result that more than two interkinesis nuclei are formed. Fig. 1 *e* shows the first anaphase: five chromosomes are moving towards one pole, two towards the other, the spindle here does not lie in the middle of the PMC. Fig. 1 *d* shows an interkinesis nucleus with three chromosomes at one pole and two dwarf nuclei at the other, the rest of the chromosomes are lagging at the equator of the spindle. In Fig. 1 *f* as many as six interkinesis nuclei can be seen, two large and four small ones. The great dispersion of univalents when interkinesis sets in is striking in this form.

The second division thereupon commences. The previously undivided centromeres are then divided, and the chromatids are separated normally. When there are several interkinesis nuclei, however, all are divided. During the second metaphase it is rather easy to count the chromosomes on the different plates. Highly varying conditions exist. Fig. 1 *g* shows the division of a restitution nucleus. But, for instance, the following chromosome numbers were observed on the plates during the second metaphase: 12, 11 + 1, 10 + 2, 9 + 2 + 1, 9 + 1 + 1 + 1, 8 + 4, 8 + 3 + 1, 8 + 2 + 2, 8 + 1 + 1 + 1 + 1, 7 + 5, 7 + 3 + + 2, 7 + 2 + 1 + 1 + ½ + ½, 6 + 4 + 2, and so on.

Of the plants examined, 2147/771 had larger PMCs and larger univalents than 3234, 3236, 3239/771. It had more often a bivalent. In 3239/771 a bivalent was never observed. Fig. 1 *b* and *c* are from 2147/771.

The sporads that are formed naturally vary much in appearance. Dyads are rather common, and pure dyads as well as such having one or two dwarf-cells in addition to the two large ones constituted 28 per cent. of the total number of sporads. No doubt the pollen-grains formed from these large grains have more than three lobes. For, as in *Oenothera*, diploid forms of *G. Whitneyi* have 3-lobed grains, whereas tetraploid forms have 4-lobed, the shape of the grains depending upon their chromosome number. The rest of the sporads consisted for the most part of polyads with more than four pollen-cells. In the tetrads the four cells are frequently of different sizes, suggesting different chromosome numbers.

The small-flowered *ff* type to which the Santa Rosa monosomics gave rise is, thus, a nullisomic. Its formation is attributable to the fact that, in these monosomics, egg-cells as well as pollen-grains with six chromosomes are vital. Since nullisomics show a frequency of more than 25 per cent., 6-chromosome pollen-grains must function often. On account of the elimination of the univalent during meiosis the

monosomic also forms more pollen-grains with six than with seven chromosomes. It would also seem that a deficient number of 14-chromosome plants are formed, judging from the statement that the great majority of plants with petal spot are partially pollen-sterile, i. e. monosomics. It is rather peculiar that n—1 grains are so vital here. In several plants, e. g. *Datura*, the loss of even a small part of a chromosome brings about abortion of the grain.

Now that the vitality of the n—1 gones has allowed of a nullisomic actually being produced, it is remarkable that it does not consist of a stunted type, as might be expected when one of the seven chromosomes of the set is missing. The feeble development of the stamens in the fully developed flowers is evidently associated with the great sterility of the pollen, which in its turn is a consequence of the considerably disturbed meiosis. Moreover, this nullisomic seems to be entirely female-sterile, HIORTH not having obtained a single seed in spite of extensive crossings. Judging from some rather large ovaries that were examined development appears to have been retarded, and it looks as if an embryo sac is only rarely formed in the ovule. Abortion of megaspore mother-cells frequently occurred, and dyads, which were evidently dead, were also observed. The appearance of dyads suggests that asyndesis also occurs in the ovules.

As the root-tips were not studied, the missing chromosome cannot be identified, a reliable identification meeting with great difficulties in *G. Whitneyi* (cf. HÅKANSSON, 1942). Genetically it seems determinable, for all the nullisomics are without petal spot, while all the non-nullisomics have such. Besides the gene *F* the missing chromosome has a gene for flower-size. It also appears to possess a dominant gene (or genes) for asyndesis. Should this gene be absent, asyndesis occurs (nullisomics); if it is present in merely one chromosome, as in monosomics, it manifests itself in a somewhat weakened pairing with the appearance of two extra univalents. In *G. Whitneyi* several cases have been encountered of partial or almost complete asyndesis, and it therefore seems probable that genes for syndesis are present in several chromosomes.

Nullisomes do not appear to be known in any diploid form. In hexaploid *Avena* and *Triticum* 40-chromosome plants are known, these often being dwarfs, some showing asyndesis. SEARS (1941) pollinated haploid *T. vulgare* with diploid pollen and obtained numerous aberrants, among them monosomics out of which nullisomics were then got. Seven nullisomics were obtained. They presented a very different

appearance and had different fertility. Two were completely male-sterile, one quite female-sterile, while four were partially both male- and female-fertile. Type III showed partial asyndesis. In this connexion attention may be called to *Viola Orphanidis* (CLAUSEN, 1930), although there it does not seem to have been a question of nullisomy. According to CLAUSEN the genus *Viola* has, among others, a 10-series, several species having n = 10 chromosomes. A small group of species has n = 11, these appearing »closest related to the pansies of the 10-series and may long ago have arisen from originally 10-chromosome violets by duplication of one chromosome». *V. Orphanidis* is closest related to *V. cornuta* and *V. orthoceras*, which have n = 11 chromosomes, and according to CLAUSEN this number is also the correct one for *V. Orphanidis*, although the latter occurs in individuals with 22, 21 and 20 chromosomes. Apart from the number of chromosomes these may resemble each other to a large extent.

HORSE MT. × BREMEN, F_2.

Morphologically Horse Mt. is a distinctive race but may none the less exhibit rather different chromosomal arrangements during the first metaphase. Plants were observed with 6-ring + 4 II, 7 II, 4-ring + + 4 II + I, finally, with 6 II + I, and the monosomics were partially pollen-sterile (HIORTH, 1942; HÅKANSSON, 1942). Plants out of a cross Horse Mt. × Bremen were rather pollen-sterile, and two plants that were examined were monosomics. The progeny have now been examined of the monosomic F_1 plants, viz. plants from the families S 662—664—1942. The F_2 contains a highly sterile type with green stem and small whitish flowers; nearly all the pollen-grains have more than three lobes but are sterile. This type might very well be a nullisomic. The rest of the plants have a red stem and most of them are partially pollen-sterile. Cytologically there are at least three types.

Of the plants with a red stem, one had 14 chromosomes and three had 13. The former had thin anthers, and only little of the material could be studied. In some PMCs seven bivalents were observed, but a certain amount of asyndesis occurs, so that two or four univalents may be formed. The monosomic plants had the 4-ring + 4 II + I arrangement. As a rule, however, there was an open chain in place of a ring. These plants also showed some asyndesis, one to four new univalents having been observed. It was the chromosomes of the chain that seemed most frequently to exhibit asyndesis. The behaviour of the constantly

occurring univalent was studied (cf. HÅKANSSON, 1940 a, pp. 16—20; 1941). Only rarely did its centromere divide at the first anaphase and its chromatids pass each to its pole. It was found in such cases that it had a submedian centromere. Usually the centromere remained undivided. The univalent then formed its own small interkinesis nucleus. During interkinesis the chromatids of the univalent were often well separated, but were held together by the undivided centromere. Thus the univalent had an X-form. The centromere divided at the second anaphase, but on account of its submedian position it was not possible to determine whether it was divided in the transverse or in the longitudinal direction of the chromosome, the former mode being so-called misdivision of centromere. A determination of its manner of division would here have been of great interest. On a few occasions a fragmentation was observed at the first division, a portion of one of the chromatids being parted from the rest and remaining in the plasm.

Five plants of the sterile type with a green stem were studied. This type shows analogies to the nullisomics from Santa Rosa 6, and it was therefore surprising to find 13 chromosomes. Like the nullisomics, all five plants showed complete asyndesis. The meiotic divisions exhibited some differences as compared with those of the nullisomics. In some prophase nuclei it could be determined with tolerable certainty that the chromosomes were also unpaired at early »diakinesis». A split that separated the chromatids could be seen in the chromosome. These stages are always very indistinct in *G. Whitneyi*, which is remarkable in view of the fact that in other *Godetia* species they are very distinct. Fig. 2 *a* shows the 13 univalents in the PMCs. They are of different sizes. In this form the univalents did not display so strong a tendency to form an equatorial plate as in the nullisomics. Certainly a plate was formed in many PMCs (Fig. 2 *d*), but no attempt at any such formation seems to have been made in more than one-half of the PMCs. This is undoubtedly the reason that a restitution nucleus is formed much more often in this form than in the nullisomic (Fig. 2 *c*). At the formation of the nuclear membrane one to three chromosomes often come to lie outside the membrane, and hence during interkinesis the PMC often has one to three small nuclei besides the large one (Fig. 2 *b*). During interkinesis at least 80 % of the PMCs contain one large nucleus or one large nucleus along with dwarf nuclei. Rarely there were found two nuclei of approximately the same size, formed as a result of an anaphase distribution of the univalents into two polar groups. More commonly, anaphase led to a marked dispersion of the

univalents, so that several interkinesis nuclei were formed. This was the commonest type of interkinesis in the nullisomic.

In the restitution nucleus of the haploid *Whitneyi* type usually only one chromosome develops a nucleolus; sometimes, however, a nucleolus is developed by two chromosomes, though not by more. Here

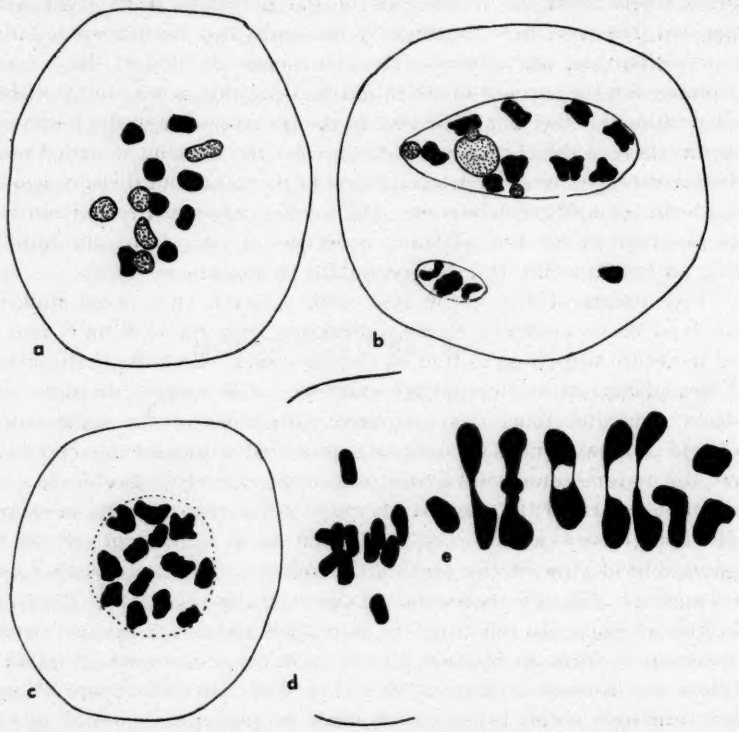

Fig. 2. Horse Mt. × Bremen, F_2. — *a*—*d*, an asyndetic form. — *a*: 13 I. — *b*: interkinesis. — *c*: a restitution nucleus is formed. — *d*: the univalents form an equatorial plate. — *e*: a plant with 14 chromosomes, 5 II + 4 I. — × 2250, *e* × 3800.

the observation was made that in the large interkinesis nuclei it is not uncommon for three or four chromosomes to form nucleoli (Fig. 2 *b*). This confirms the assumption that the genome contains two nucleolus-forming chromosomes.

The second division was not closely studied in this form. The result of the meiotic division was that more than 80 per cent. of the sporads were dyads or dyads with dwarf cells. Most of the remaining 20 per cent.

were polyads with more than four (often rather many) pollen-cells. There were thus formed many pollen-cells with 10—13 chromosomes, but also many with few chromosomes. Pollen-cells with intermediary numbers were comparatively rare. Probably the dwarf-cells disappear early, and hence the great majority of fully developed pollen-grains contained 10—13 chromosomes. This explains why all or almost all pollen-grains have more than three lobes. According to HIORTH they are nevertheless sterile.

The Horse Mt. type with small flowers and extremely high sterility is accordingly no nullisomic. Moreover, it does not occur in a frequency of 25 % or more, but has a considerably lower frequency, only a few per cent., though what frequency is not yet exactly established. At least 5 but no more than 12 plants of 190 in the families S. 662—664 had green stems. Some points of resemblance which it bears to the nullisomic are due to complete asyndesis, as a result of which pollen-grains with 7 chromosomes are very rarely produced. The univalents, however, form here an equatorial plate with a following anaphase more seldom than in the nullisomic, which may merely be due to the fact that interkinesis possibly sets in earlier and a plate has not time to form. In any case the result is that the pollen picture differs to some extent from that of the nullisomic with the multilobed pollen-grains in very great predominance. However, the Horse Mt. type may be said to agree with the nullisomic in at least two characters, viz. occurrence of complete asyndesis and small flowers. As other morphological characters distinguish it, i. e. the green stem and the whitish flowers, its origin cannot be accounted for on the basis of a recessive gene for asyndesis. Probably the matter lies thus, that nullisomy is also present here, either in respect of a whole chromosome or else of a fragment, perhaps an arm from a chromosome.

If this type is nullisomic for that chromosome which is a univalent in the monosomic, it must be trisomic for another chromosome. On account of asyndesis this cannot be observed, since no trivalent can be formed here. The monosomics show, as was mentioned, a weak asyndesis that seems especially to involve the chromosomes of the chain. Undoubtedly there can be formed in the monosomics a tetrad nucleus that has chromatids from two random univalents of this kind but none from the ordinary univalent. Fusion with an ordinary 6-chromosome gamete would give a zygote that is trisomic for one chromosome, nullisomic for another. It is a question, however, whether such 7-chromosomal gametes are formed so often as this explanation

of the formation of the asyndetic plants would require. This does not seem to be the case.

The other explanation would imply that in the monosomics the univalent underwent such a change comparatively often that a segment was lost. A gamete with the changed chromosome would then fuse with a 6-chromosome one and the zygote become nullisomic for the lost segment. Still, it is clear that the observed fragmentation of the univalent takes place too rarely to come into consideration as a basis for explanation.

In *Triticum vulgare* a chromosome has been encountered which fairly constantly has a chiasma formed between its two arms (HÅKANSSON, 1932). This chromosome was found in subcompactoid and compactoid types that had arisen from monosomic speltoid B-heterozygotes which had one univalent. It was supposed that the peculiar so-called co-chromosome had arisen through crossing-over between duplicated portions in the univalent of the monosomic. Another explanation has, however, been given (DARLINGTON, 1939, 1940; RHOADES, 1940). In *Fritillaria kamtschatkensis* DARLINGTON found univalents that during the first anaphase divided transversely instead of longitudinally, so-called misdivision of centromere. As a result two telocentric chromosomes with terminal centromere were formed. In the next division the chromosome arm was divided as usual, but not the centromere. The result was a new chromosome with the centromere in the middle and the two arms identically alike, a so-called iso-chromosome. The origin of an iso-chromosome at mitosis in the pollen-grain after misdivision at the second anaphase has been established by DARLINGTON. RHOADES found a telocentric chromosome in maize plants. It was instable, and in the next generation an iso-chromosome was found. Misdivision must have taken place at the second division or at the pollen-grain mitosis. As the telocentric chromosome was characterized by definite genes, it was possible to demonstrate that each of the arms of the iso-chromosome corresponded to the telocentric chromosome. These observers have considered it probable or certain that the co-chromosome in *Triticum* has arisen through misdivision and is therefore an iso-chromosome. The same is thought to apply to the extra chromosome in secondary trisomics in *Datura*.

Misdivision has been observed in haploid rye. During the second division there were found new chromosomes that must have arisen in this manner (LEVAN, 1942). Misdivision has also been observed at the first anaphase in *G. Whitneyi*, e. g. in the haploid (HÅKANSSON, 1940 b) and

in a mutant with narrow leaves (HÅKANSSON, 1940 a). But, as was pointed out above, its occurrence has not admitted of being definitely established in monosomic types of Horse Mt. This does not signify, of course, that it does not occur. The assumption that the asyndetic type has an iso-chromosome and is therefore nullisomic for one chromosome-arm, disomic for the other has, accordingly, not been proved. No chiasma formation within this chromosome, as within the co-chromosome, is to be expected, since asyndesis exists.

Addendum. — The following description of the nullisomic type from Santa Rosa 6 has been sent to me by Dr. HIORTH. Apparently the monosomics have normal size, good vitality, and are normal in almost every way. Respecting their pollen-sterility it may be stated that the two semi-sterile F_1 plants have 39 and 45 % poor pollen respectively. The fertil F_1 plant, which is undoubtedly not a monosomic, has only 4 %. The nullisomics are somewhat dwarflike, a fact that is manifested in their low growth, slender branches and small flowers; they are however in no way stunted and have rather good vitality, although they are sterile. The ovary is strikingly long with eight ribs but with only few developed ovules. HIORTH states that in appearance the nullisomic gives the impression of being a new species. As already mentioned, it occurs in high frequency. For instance, in S 771—1942 the segregation was 48 normal : 34 small-flowered plants.

SUMMARY.

In the progeny from monosomic plants of the race Santa Rosa 6 crossed with Bremen there occurs with very high frequency a nullisomic that possesses such extremely distinctive characters as to suggest a new species. It shows complete asyndesis, with almost invariably 12 univalents. A restitution nucleus is rather often formed, resulting in about 28 % dyads. However, the plant is quite sterile.

In the progeny from monosomic plants of the race Horse Mt. crossed with Bremen there occurs in some few per cent. a morphologically very characteristic type. Like the monosomics, however, it has 13 chromosomes but shows complete asyndesis with 13 univalents. A restitution nucleus is very often formed, and dyads are developed in a proportion of more than 80 %. The plant is quite sterile. No doubt, this type is nullisomic in respect of a fragment of a chromosome, probably an arm, and has arisen through misdivision of the centromere of the univalent in the monosomic parent plant.

LITERATURE CITED.

1. CLAUSEN, J. 1930. Male sterility in *Viola Orphanidis*. — Hereditas XIV.
2. DARLINGTON, C. D. 1939. Misdivision and the genetics of the centromere. — Journ. of Genetics 37.
3. — 1940. The origin of iso-chromosomes. — Journ. of Genetics 39.
4. GOODSPEED, A. H. and AVERY, P. 1929. The occurrence of chromosome variants in *Nicotiana alata* LK. et OTTO. — Proc. Nat. Acad. sci. Wash. 15.
5. HIORTH, G. 1942. Zur Genetik und Systematik der *amoena*-Gruppe der Gattung *Godetia*. — Zschr. f. ind. Abst.- und Vererbungslehre, Bd. 80.
6. HÅKANSSON, A. 1932. Zytologische Studien an compactoiden Typen von *Triticum vulgare*. — Hereditas XVII.
7. — 1940 a. Die Meiosis bei verschiedenen Mutanten von *Godetia Whitneyi*. — Lunds Univ. Årsskrift, N. F. Avd. 2, Bd. 36, Nr. 5.
8. — 1940 b. Meiosis in haploiden Pflanzen von *Godetia Whitneyi*. — Hereditas XXVI.
9. — 1941. Zytologische Beobachtungen an Kreuzungen zwischen *Godetia deflexa* und *G. Whitneyi*. — Botaniska Notiser.
10. — 1942. Zytologische Studien an Rassen und Rassenbastarden von *Godetia Whitneyi* und verwandten Arten. — Lunds Univ. Årsskrift, N. F. Avd. 2, Bd. 38, Nr. 5.
11. LAMMERTS, W. E. 1932. Inheritance of monosomics in *Nicotiana rustica*. — Genetics 17.
12. LEVAN, A. 1942. Studies on the meiotic mechanism of haploid rye. — Hereditas XXVIII.
13. RHOADES, M. M. 1940. Studies of a telocentric chromosome in maize with reference to the stability of the centromere. — Genetics 25.
14. SEARS, E. R. 1941. Nullisomes in *Triticum vulgare*. — Abstracts of papers pres. to the 1940 meetings of the Gen. Soc. of America, Genetics 26.

ABSTRACTS — KURZE MITTEILUNGEN

ANTERO VAARAMA: Beobachtungen über die Meiose bei einigen Anthropochoren.

In der Literatur gibt es zahlreiche experimentelle Untersuchungen, in denen der Einfluss extremer Temperaturen auf die Reifeteilung der Pflanzen behandelt wird. Sie gehen allgemein auf die Feststellung aus, dass als Folge solcher Einflüsse mannigfache Störungen im Verlauf der Reifeteilung eintreten. Von den diesbezüglichen Arbeiten seien hier nur die von SAKAMURA (1920), BORGENSTAM (1921), DE MOL (1923), MICHAELIS (1925) und MICHAELIS und v. DELLINGSHAUSEN (1933) erwähnt.

Dagegen enthält die Literatur nur sehr wenig sowohl experimentell wie in der Natur selbst erzielte Befunde darüber, welche die höchsten bzw. niedrigsten Temperaturen sind, innerhalb der sich die Meiose der Pflanzen normalerweise vollzieht.

Als bescheidener Beitrag zu den spärlichen Angaben mögen folgende Beobachtungen über die Meiose einiger noch ganz am Ende der Vegetationsperiode blühenden Ruderatpflanzen dienen.

Die Beobachtungen betreffen die Arten *Capsella bursa pastoris*, *Senecio vulgaris*, *Thlaspi arvense* und *Fumaria officinalis*, die in den Kulturen der Gartenbauanstalt Lepaa (Finnland), etwa 20 km von der Stadt Hämeenlinna, als gemeine Unkräuter wachsen. Von den zwei erstgenannten Arten wurde Knospenmaterial am 31. X. 1939 um 14h/00 und von den beiden letzteren drei Tage später um 12h/30 fixiert. Über die Temperaturverhältnisse am Materialeinsammlungstag und den vorhergehenden Tagen gibt die beigefügte Tabelle, deren Zahlen an der 100 m vom Standort der Pflanzen gelegenen meteorologischen Beobachtungsstation in der Zeit vom 25. X. bis zum 3. XI. erhalten wurden, Auskunft.

Dem Materialeinsammlungszeitpunkt am nächsten wurde also an der Beobachtungsstation am 31. X. die Temperatur + 0,7° C und am 3. XI. ent-

1939	Temperatur			Maximum	Minimum	Minimum an der Erdoberfläche um 7 Uhr
	7 Uhr	15 Uhr	21 Uhr			
Okt. 25.	3,4	5,0	1,8	5,4	1,8	3,0
26.	— 1,0	3,8	— 2,0	4,8	— 3,2	— 6,3
27.	— 5,8	0,6	— 1,4	3,2	— 6,0	— 10,0
28.	— 2,0	— 1,7	— 2,7	— 1,3	— 3,0	— 2,0
29.	— 1,3	1,0	0,3	1,3	— 3,2	— 3,0
30.	— 0,5	— 1,3	— 2,0	0,8	— 2,2	— 2,0
31.	— 2,2	0,7	0,2	0,9	— 3,7	— 3,0
Nov. 1.	— 1,8	— 1,0	— 2,0	— 0,1	— 1,0	— 1,0
2.	— 3,0	— 2,0	— 2,2	— 1,6	— 3,3	— 5,0
3.	— 3,9	— 3,8	— 5,0	— 1,6	— 5,0	— 7,0

sprechend — 3,8° C gemessen. Doch war die Wärmestrahlung der Sonne an beiden Tagen an offenem Standort immerhin so gross, dass die wirkliche Temperatur in der unmittelbaren Nähe der Pflanzen im Augenblick der Fixierung am erstgenannten Tage um 14h/00 + 3,0° C und am zweitgenannten um 12h/30 + 1,5° C betrug. Die Tabelle soll in erster Linie zeigen, bei welchen Temperaturen sich die fixierten Knospen zuvor entwickelt hatten. Die letzte Spalte, die an der Erdoberfläche gemessene Wärmeminima angibt, zeigt wohl am besten gerade die am Standort der Pflanzen herrschenden Temperaturextreme. Es sei erwähnt, dass zu der gleichen Zeit, als das Material eingesammelt wurde, die Knospen des bei der Anstalt zweitmalig in Blüte getretenen *Melampyrum nemorosum*, einiger Kultursorten der Himbeere sowie einiger kultivierten *Rosa*-Arten völlig erfroren waren.

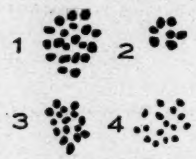

Fig. 1. 1. *Senecio vulgaris*, n = 20. — 2. *Thlaspi arvense*, n = 7. — 3. *Capsella bursa pastoris*, n = 16. — 4. *Fumaria officinalis*. n = 16. — Vergr. 2000 ×.

Die Fixierung erfolgte in Chrom-Essig-Formalinlösung nach NAWASCHIN—WEBBER, die Färbung fand nach dem üblichen Jod-Kristallviolettverfahren statt.

Bei der mikroskopischen Untersuchung konnten in den Pollenmutterzellen sämtlicher Arten ziemlich reichlich verschiedene Stadien der Reifeteilung festgestellt werden. Ferner ergab sich, dass sämtliche Teilungen trotz der niedrigen Temperaturen, unter deren Einwirkung sich die Pollenmutterzellen während ihrer Entwicklung befunden hatten, keinerlei Störungen aufwiesen. Die Pollenkörner waren bei sämtlichen Arten normal entwickelt.

Die gemachten Beobachtungen führen zu der Feststellung, dass die Mindesttemperatur, bei der die Meiose in Pollenmutterzellen noch ungestört abläuft, bei sämtlichen untersuchten Arten sehr niedrig liegt, bei *Capsella bursa pastoris* und *Senecio vulgaris* zumindest unter + 3,0° C, bei *Thlaspi arvense* und *Fumaria officinalis* wenigstens unter + 1,5° C.

Alle untersuchten Arten sind ökologisch miteinander verwandt. Bei sämtlichen handelt es sich um Anthropochoren, gemeinste Ruderatpflanzen, die sich durch reichlich fruktifikative Vermehrung auszeichnen. Das niedrige Temperaturminimum der Meiose ist offenbar eine wichtige damit im Zusammenhang stehende Eigenschaft, die es den Pflanzen ermöglicht, noch bis zum äussersten Extrem der Vegetationsperiode vollwertigen Samen hervorzubringen.

Was die Chromosomenzahlen der untersuchten Arten (Fig. 1) betrifft, so entsprechen die gefundenen Zahlen, n = 16 für *Capsella bursa pastoris*, n = 7 für *Thlaspi arvense* und n = 20 für *Senecio vulgaris*, den zuvor für diese Arten festgestellten [siehe LÖVE und LÖVE (1942)]. Dagegen weicht die bei *Fumaria officinalis* sowohl in der Meiose (Fig. 1) wie in der Mitose ermittelte Chromosomenzahl n = 16 völlig von der Zahl x = 7 ab, die sowohl WULFF (1937) wie SUGIURA (1940) als die die fragliche Gattung kennzeichnende Grundzahl angeben. Ich hoffe später näher auf die Ursachen dieses Unterschiedes eingehen zu können.

Damit bezüglich der Priorität keine Unklarheit entsteht sei hier erwähnt, dass zwei finnische Forscher, T. OKSALA und O. V. LUMIALA, schon vor mir eine Untersuchung der ökologischen Bedeutung des Temperaturminimums der Meiose unter natürlichen Verhältnissen auf sehr breiter Basis in Angriff ge-

nommen haben. Infolge der waltenden Zustände ist die Veröffentlichung ihrer Resultate jedoch verzögert worden.

Höhere staatl. Gartenbauschule, Lepaa, Finnland.

Zitierte Literatur.

1. Borgenstam, Elsa. 1921. Zur Zytologie der Gattung *Syringa* nebst Erörterungen über den Einfluss äusserer Faktoren auf die Kernteilungsvorgänge. — Ark. f. Bot., 17, n:o 15.
2. de Mol, W. E. 1923. Duplication of generative nuclei by means of physiological stimuli and its significance. — Genetica, 5.
3. Löve, A. and Löve, Doris. 1942. Chromosome numbers of Scandinavian plant species. — Bot. Not.
4. Michaelis, P. 1925. Über den Einfluss der Kälte auf die Reduktionsteilung von *Epilobium*. — Planta, 1.
5. Michaelis, P. und v. Dellingshausen, M. 1936. Entwicklungsgeschichtlich-genetische Untersuchungen an *Epilobium*. VII. Experimentelle Untersuchungen über die Beeinflussung der Pollenfertilität unter besonderen Berücksichtigung der Plasmonwirkung. — Jahrb. f. Bot., 82.
6. Sakamura, T. 1920. Experimentelle Studien über die Zell- und Kernteilung mit besonderer Rücksicht auf Form, Grösse und Zahl der Chromosomen. — Journ. Coll. Sci. Imp. Univ. Tokyo, 39.
7. Sugiura, T. 1940. Chromosome studies on Papaveraceae with special reference to the phylogeny. — Cytologia, 10.
8. Wulff, H. D. 1937. Chromosomenstudien an der schleswig-holsteinischen Angiospermen-Flora. I. — Ber. Deutsch. Bot. Ges., 55.

Robert Lamm: Notes on an octaploid *Solanum punæ* plant.

Among the tuber-forming *Solanum* species *Solanum tuberosum* L. has been most thoroughly cytogenetically investigated. There are different views as to whether this tetraploid ($2n = 48$) species should be considered to be an auto- or allopolyploid. In order to probe into this problem the author has tried to produce artificial autopolyploid *Solanum* plants of related species in order to compare the meiotic behaviour of such plants with the meiosis in *S. tuberosum*. For these experiments the diploid species *S. Rybinii* Juz. et Buk. ($2n = 24$) and the tetraploid species *S. punæ* Juz. ($2n = 48$) were chosen.

S. punæ is a wild Peruvian species belonging to the section *Acaulia* of the tuber-forming *Solanum* species. This section is systematically far isolated from the section *Tuberosa* (Bukasov, 1933) to which the two other species here mentioned, *S. Rybinii* and *S. tuberosum*, belong. The plants of *S. punæ* are prostrate and rosette-forming. Tubers are only formed under short-day conditions. *S. tuberosum* is always more or less pollen-sterile, while the pollen of *S. punæ* contains practically no dead or empty grains. The fruit and seed setting of *S. punæ* is also very abundant. In *Solanum acaule* Bitt. ($2n = 48$) meiosis is characterized by a strict pairing into bivalents (Propach, 1937). This statement according to my observations is also applicable to *S. punæ*,

whereas in *S. tuberosum*, besides bivalents, univalents, trivalents and quadrivalents are formed.

Young seed plants of *S. punæ* were treated with lanoline paste containing 0,1 per cent colchicine. By this method an octaploid (2n = 96) *S. punæ* plant was obtained in the summer of 1942. This plant differed from its tetraploid relatives by broader and thicker leaves, larger flowers and a rougher appearance. In the microscope about 66 per cent of the pollen-grains appeared to be fertile. The seed setting of self-pollinated flowers was fairly good. It also seems as if it should be easier to obtain successful crosses between octaploid *S. punæ* and *S. tuberosum* than between tetraploid *S. punæ* and *S. tuberosum*, although the two parents in the last-mentioned cross have the same chromosome number. In practical breeding such crosses are of some importance because of the frost resistance of *S. punæ*. The vigour and fertility of this octaploid *S. punæ* plant is remarkable, since artificial octaploids of *S. tuberosum* as a rule are rather weak and sterile.

In octaploid *S. punæ* about seven quadrivalents were observed during diakinesis and first metaphase of the meiotic division. The greater part of the chromosomes formed bivalents. A few trivalents and univalents were also observed. This behaviour may be explained by a strong tendency to two-by-two pairing of the same kind as has been found in *Phleum* (MÜNTZING and PRAKKEN, 1940). Before these meiotic conditions of the octaploid were known, one could suspect the strict bivalent pairing of the normal tetraploid *S. punæ* to have the same cause. If, however, the four genomes of the tetraploid were homologous it is likely that here, too, a few quadrivalents would be formed just as in the octaploid *S. punæ* plant. A critical test of these speculations would be afforded by observations of the meiotic conditions in haploid *S. punæ*, but such a plant has so far not been found. In the progenies of *S. tuberosum* and of closely related tetraploid hybrids, diplo-haploid (2n = 24) plants have been found showing twelve bivalents during meiosis (LAMM, 1938; IVANOVSKAJA, 1939). In haploid *S. punæ* there would probably be no or very weak chromosome pairing.

When observing the pollen-grains of the octaploid *S. punæ* plant in the microscope, a great number of grains with four germ-pores are found. In respect of this character it agrees with *S. tuberosum* and not with tetraploid *S. punæ*, the pollen-grains of which have three germ-pores just as *S. Rybinii* and other diploid *Solanum* species.

All these circumstances indicate that the type of polyploidy is not the same in tetraploid *S. punæ* as in tetraploid *S. tuberosum*. The former has more the characters of an allotetraploid, the latter of an autotetraploid species. The evolution of polyploid derivatives among the tuber-bearing species of the genus *Solanum* may thus have followed different lines.

Alnarp in October, 1942.

Literature cited.

1. BUKASOV, S. M. 1933. The potatoes of South America and their breeding possibilities. — Suppl. 58, Bull. Appl. Bot. Genet. and Pl. Breed., Leningrad.
2. IVANOVSKAJA, E. V. 1939. A haploid plant of *Solanum tuberosum* L. — Comptes

Rendus (Doklady) de l'Académie des Sciences de l'URSS., Vol. XXIV, No. 5: 517—520.

3. LAMM, R. 1938. Notes on a haploid potato hybrid. — Hereditas XXIV: 391—396.

4. MÜNTZING, A. and PRAKKEN, R. 1940. The mode of chromosome pairing in *Phleum* twins with 63 chromosomes and its cytogenetic consequences. — Hereditas XXVI: 463—501.

5. PROPACH, H. 1937. Cytogenetische Untersuchungen in der Gattung *Solanum*, Sect. *Tuberarium*. II. Triploide und tetraploide Artbastarde. — Zeitschr. f. ind. Abst.- u. Vererb.-lehre, Bd. LXXIII, H. 1: 143—154.

J. A. BÖÖK: Low temperature and cleavage in *Triton taeniatus*.

The induction of haploidy in the salamander *Triton taeniatus* by means of cold treatment of unsegmented egg-cells was announced by the present writer a year ago (BÖÖK, 1941). In that paper the conception of the problem at that time was briefly outlined. The occurrence of balanced autotriploidy in *Triton taeniatus* has also been described (BÖÖK, 1940).

Since then further experiments have been undertaken to shed some light on the problem. In a series of experiments, in which 112 egg-cells were exposed within half an hour after fertilization to a temperature of + 1° and + 2° C during 6—15 hours, 39 developed into living larvae. Five of these appeared to be homogeneous triploids. Chromosome counts and measurements of nuclear size were made for different tissues (brain, epithelium of cutis, cartilage and erythrocytes). Thirty-four larvae were perfectly normal diploids. The majority of the cold treated egg-cells degenerated before or during gastrulation. The mortality of the treated series was 58 % and of the controls 11,3 %. No difference in duration of the intraovular development was found between the treated material and the controls.

There seems to be no significant correlation between duration of cold treatment, temperature (+ 1° and + 2° C) and mortality.

In another series of experiments, where the temperature treatment was set in later than half an hour after fertilization but before the first cleavage, the mortality was heavily increased. Sixty-two egg-cells were treated, and from these only 13 embryos lived after 3 days. It was possible to make a cytological examination of six of these surviving embryos. All of them showed an abnormal nuclear constitution. The chromosome numbers found in each embryo were as follows.

Embryo No.	Chromosome numbers
$7\frac{1}{2}$ I	17, 24
II	16, 24, 27
III	11, 12, 13, 14, 18, 26, 27, 31, 33
V	28 ± 2, 30 ± 2
VI	30 ± 2, 33
3 II	26, 28, 29 ± 2

Every embryo contained nuclei with different numbers of chromosomes. An abnormal great variation of nuclear size accordingly was found. It is

remarkable that, in spite of these varying chromosome numbers, there were macroscopically no signs of abnormalities in these cases.

In a third series, embryos at the stage of the neural fold were treated with low temperature (+ 2° C) for 8 hours. Cytological examination was undertaken 5 hours later. In these embryos chromosome plates were found containing, besides normal metaphase chromosomes, also chromosomes the centromeres of which had already divided without signs of repulsion (Fig. 1). A tripolar anaphase also was found. It should be noticed that in normal diploid embryos endomitotic metaphases are often met with, but in these cases the endomitotic reduplication takes place earlier and can be already observed

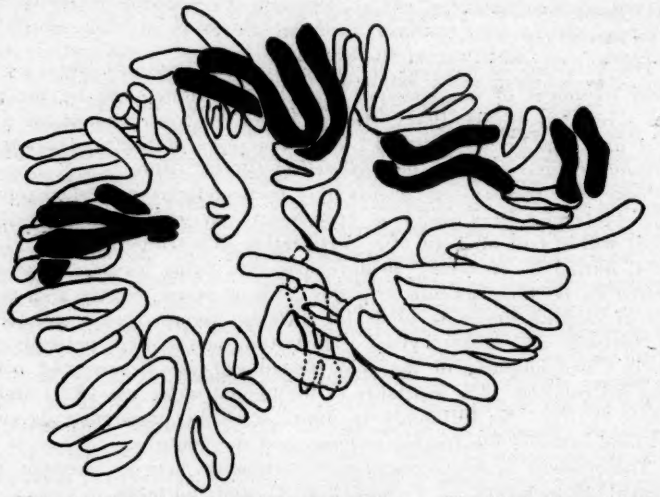

Fig. 1. Metaphase plate from cold treated embryo. The black chromosomes are divided at the centromeres.

during prophase. Thus, in the metaphase plate all the 24 chromosomes are present as pairs of chromatids, the centromere of each chromosome being completely divided.

On the basis of these observations the influence of low temperature on the unsegmented egg-cells may be explained as follows.

The essential factor is probably not minor variations in temperature. Assuming that the temperature is low enough to prevent the anaphase movements, i. e. the normal development of the spindle, the most important thing is the actual nuclear constitution of the egg-cell when the cold shock is applied. The low temperature induces a paralysis of the spindle apparatus, but the centromeres and the centrosomes seem either to be unaffected or to recover quicker. When the cold shock strikes the egg-cell within half an hour after fertilization, at least two alternatives may be followed. If it is in the second metaphase stage, which is always the case when the sperm is penetrating, a metaphase reduplication takes place. Owing to the paralysis

of the spindle apparatus the anaphase movements are inhibited and a restitution nucleus with 24 chromosomes is formed. Later on, this diploid nucleus is fertilized by the haploid sperm nucleus and a balanced autotriploid embryo develops.

The other alternative to be mentioned here is that the egg-cell has reached the stage when the second polar body is already formed. The sperm nucleus and the egg nucleus are still lying apart. The cold shock, applied at this time, will inhibit movements and subsequent fusion of the pronuclei. The sperm nucleus, which has a centrosome, would then divide alone and development proceed with the haploid set of chromosomes. A somewhat similar explanation, which however labours with minor temperature differences, has been offered by FANKHAUSER and GRIFFITHS (1939). Other alternatives probably lead to the destruction of the zygote.

This explanation, considering the fact that haploidy and triploidy are induced under the same experimental conditions, is also supported by the findings of GRIFFITHS (1941). Nor in his experiments on *Triturus viridescens* were triploids or haploids raised when the cold shock was set in later than half an hour after fertilization.

Embryos containing variable chromosome numbers were formed in these experiments when the cold shock hit the egg-cell after normal fertilization but before the first cleavage of the zygote. At this point the zygote must have been at the stage of its first somatic metaphase. The spindle apparatus is paralysed and during this time there occurs metaphase reduplication. The consequence is that the next division becomes multipolar. Thus all possibilities are realized for the formation of nuclei with different chromosome numbers and embryos that could be called multiple aneuploids.

A more detailed description of these experiments will be given in a later paper, in which the meiosis in triploid *Triton taeniatus* will also be described and discussed.

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Literature cited.

1. BÖÖK, J. A. 1940. Triploidy in *Triton taeniatus* LAUR. — Hereditas XXVI.
2. — 1941. Induction of haploidy in a cold treatment experiment with egg-cells of the salamander *Triton taeniatus*. — Kungl. Fys. Sällsk. i Lund Förh. Bd. 11. Nr. 12.
3. FANKHAUSER, G. and GRIFFITHS, R. 1939. Induction of triploidy and haploidy in the newt *Triturus viridescens* by cold treatment of unsegmented eggs. — Proc. Nat. Acad. of Sci. Vol. 25. No. 5.
4. GRIFFITHS, R. 1941. Triploidy (and haploidy) in the newt *Triturus viridescens*, induced by refrigeration of fertilized eggs. — Genetics, Vol. 26, No. 1.

FREDRIK NILSSON and ENAR ANDERSSON: Polyploidy in the genus *Medicago*.

In connection with plant breeding at Svalöf colchicine treatments of lucerne were made for the purpose of producing new polyploid types. Three

different strains were treated, of which two belonged to *Medicago sativa* and the third was a population from the cross *M. sativa* × *falcata*. Several seedlings of all strains showed signs of »colchicine-effect» and a few of them were found to be polyploids in single branches. The untreated material had the somatic chromosome number 32 but is called diploid, and in agreement with this the number 48 is called triploid, and the number 64 tetraploid. The seedlings were planted in pots and vegetatively propagated by cuttings. Supposed tetraploid branches of the treated plants were crossed with each other. Some seeds developed and gave rise to both triploid and tetraploid seedlings, due to the chromosome number in the crossed flowers. All the cuttings and seedlings were investigated as to chromosome number. Of the cuttings some were found to be diploid, others tetraploid, and in some cases both the diploid and the tetraploid number was counted in different roots. Several tetraploids belonged to the same mother plant and had the same genotypical constitution. In two cases diploid and tetraploid cuttings were obtained from the same plant and differed only in the chromosome number and characteristics dependent on the chromosome doubling.

For comparison between types with different chromosome number the material was studied in respect of several characters. Exact measurements were made on the length of stomata, the length and width of the flowers and the tubes, and so on. All individuals were measured and of each individual ten observations were taken.

Of particular interest is the different reaction of two genotypes regarding chromosome doubling. These genotypes were called *a* and *b*, of each of which four diploid and four tetraploid cuttings were investigated. Genotype *a* was a pure *sativa* type of Bulgarian origin, while genotype *b* originated from Ultuna lucerne and was somewhat intermediate between *sativa* and *falcata*. When transferred to tetraploidy all the characters were intensified and increased in genotype *a*, while genotype *b* was found to be weaker in tetraploid than in diploid condition. The differences are in most cases significant and there is no doubt about the difference of behaviour between the two genotypes, the one showing gigas-signs after chromosome doubling and the other being weaker as tetraploid than as diploid. The stomata and pollen grains are in genotype *a* larger in the tetraploid cuttings, whereas genotype *b* showed no significant difference.

The fertility was studied to some extent in 1941. The pollen fertility did not seem to be reduced in the tetraploids, but the seed setting was very weak.

The triploids showed very good vitality and intensified characters in comparison with diploids. The fertility is probably greatly reduced.

For a more detailed report on the investigations see NILSSON and ANDERSSON, 1941.

Literature cited.

1. NILSSON, FREDRIK and ANDERSSON, ENAR. 1941. Polyploidi hos släktet *Medicago*. — Sv. Utsädesf. Tidskr. Vol. LI.

ERIK ÅKERBERG: Further studies of the embryo- and endosperm-development in *Poa pratensis*.

In an earlier paper (ÅKERBERG, 1942) I was able to show in one apomictic strain of *Poa pratensis* that the embryo develops by autonomous parthenogenesis and that the endosperm development is induced by pollination. Probably the formation of endosperm is preceded by the fertilization of the central fusion nucleus. Autonomous parthenogenesis has also been observed by TINNEY (1940), although no further experience of endosperm development was available from this species. In *Poa alpina*, however, HÅKANSSON (1943) has found, in two examined apomictic strains, autonomous parthenogenesis and induced endosperm formation with fertilization of the central nucleus. Endosperm formation failed when fertilization had not occurred. Thus, we have here a similar phenomenon to that previously observed in *Hypericum* (NOACK, 1939) and *Potentilla* (GENTCHEFF and GUSTAFSSON, 1940).

With a view to extending our experience of embryo- and endosperm-formation in *Poa pratensis* five new strains have been examined. Three of these, 929, 934, 946, were collected in Finland, the fourth, 988, in Jämtland, and the fifth was strain 5303 described in my previous paper (ÅKERBERG, l. c.). The formation of the embryo and endosperm was followed through successive fixations after anthesis had commenced (in strain 5303 fixations of earlier stages were also made) as well as through fixations of emasculated flowers with or without pollination.

Strain 5303. — In this strain, which was included in earlier investigations but was not then embryologically examined, the E. S.-development was followed from the E. M. C. As was earlier found in *Poa pratensis*, aposporous E. S.-initials concurrent with degeneration of the E. M. C. could be clearly observed here. Everything tends to show that in this strain also the E. S. is formed normally from the aposporous E. S.-initials. This also agrees with findings at the examination of the progeny of the strain.

In later stages of development the embryo proved to be multicellular while the central fusion nucleus had not had time to undergo any division. That there has most probably been an autonomous development of the embryo without fertilization is shown by the result obtained from emasculated flowers, in which multicellular embryos were also observed. No endosperm was formed in these. On the other hand, endosperm is formed in panicles after open pollination. If a fertilization of the central fusion nucleus has preceded endosperm formation, the endosperm nuclei ought to contain about 195 chromosomes after self-fertilization. This should be so because strain 5303 has $2n = \pm 78$, and as the endosperm in the E. S. from aposporous E. S.-initials gets 5n chromosomes, the number will be $5 \times \pm 39 = \pm 195$ chromosomes. One plate offered tolerably good possibilities for counting the chromosomes. The result was at least 168—170. As this number is to be regarded as a minimum, one may venture to assume that it has arisen through fertilization and not solely through the fusion of the polar nuclei. The fixed flower was derived from a panicle after open pollination, and hence selfing need not necessarily have taken place. The endosperm may therefore have obtained a number deviating from $\pm$ 195.

Strain 929. — Spikelets were fixed from isolated but not emasculated panicles after noon (at 3 and 9 p. m.) on the same day that anthesis had started as well as one and two days after this. In the three first fixations, i. e. not later than one day after the beginning of anthesis, there were observed in all flowers few- to many-celled embryos, synergids, usually one central fusion nucleus, at times two polar nuclei as well as antipodes. Two of the flowers contained twin embryos. In no case was endosperm formation observed.

The conditions were different in flowers fixed two days after the beginning of anthesis. In all of them were observed embryo-sacs in which with one exception endosperm had commenced to develop. In the older ones a distinct incipient degeneration of the antipodes could be observed. Whether endosperm development had been preceded by fertilization could not be checked.

Strain 934. — From an emasculated, non-pollinated panicle that was fixed rather late multicellular embryos as well as central fusion nuclei and antipodes but no endosperm formation were obtained in all flowers. No seed was developed in such panicles. On the other hand, evidence of endosperm formation was found in at least one flower that had been emasculated and afterwards pollinated with *pratensis*. The result of continuous fixations, carried out in the same way as for strain 929, agreed in the main with that obtained for this strain, but, in addition, certain peculiarities appeared. Here, too, there were multicellular embryos with central or polar nuclei in early fixations. But in later ones certain flowers showed a very strong growth of the antipodes, so that these almost filled the whole of the nucellar chamber. A similar phenomenon has been observed in *Poa alpina* by HÅKANSSON (l. c.), more especially in unfertilized flowers. Endosperm formation could be seen in flowers that were fixed about the same time after the beginning of anthesis as in strain 929.

Strain 946. — Flowers from only two panicles were fixed. In one panicle the flowers were emasculated but not pollinated. The fixing was done two days after the stigmas became visible. In all the flowers were multicellular embryos, mostly with central fusion nuclei, occasionally with unfused polar nuclei. No endosperm formation could be seen in any case, nor any seed formation. Commencing degeneration of the E. S., however, was observed in one flower. This was also noticed in flowers from the other fixed panicle, which had been emasculated and pollinated with *pratensis*, although in these cases the pollination had evidently yielded no result. The vigorous growth of the antipodal cells observed in strain 934 could also be noticed in some flowers.

Strain 988. — The material from this strain is very small as yet. In it, however, the occurrence of autonomous embryo development has also been established, multicellular embryos having been obtained from emasculated but not pollinated flowers. A distinct degeneration, on the other hand, could be observed in certain flowers. The fixing was done, in fact, rather a long time after the emasculation.

Summary. — Autonomous embryo development has been observed in the emasculated but not pollinated panicles of four of the five strains. It seems

most probable that the same is the case in the fifth strain, from which no emasculated, non-pollinated panicles have been examined, since here, too, the egg nucleus underwent division considerably earlier than the central fusion nucleus.

Respecting the endosperm development it may be stated that all the results from emasculation and pollination experiments as well as from embryological examination tend to indicate that it is induced. If pollen has not been available, there has been no endosperm formation. No seeds have then developed. But when pollen has been at hand, endosperm has been formed. In one case, so far the first within apomictic *Poa pratensis*, it has been established with a high degree of probability that the central fusion nucleus had been fertilized before the division.

In earlier investigations it was shown that apomixis is combined with pseudogamy in *Poa pratensis*. In view of this and of the results now submitted, it would seem justifiable to assume that autonomous embryo development with induced endosperm formation (fertilization most probable) is a general phenomenon in apomictic types of this species.

Literature cited.

1. GENTCHEFF, G. and GUSTAFSSON, Å. 1940. Parthenogenesis and pseudogamy in *Potentilla*. — Bot. Not.
2. HÅKANSSON, A. 1943. Die Entwicklung des Embryosacks und die Befruchtung bei *Poa alpina*. — Hereditas XXIX.
3. NOACK, K. 1939. Über *Hypericum*-Kreuzungen. VI. Fortpflanzungsverhältnisse und Bastarde von *Hypericum perforatum* L. — Zeitschr. f. ind. Abst.- u. Vererb.-lehre, 76.
4. TINNEY, F. W. 1940. Cytology of parthenogenesis in *Poa pratensis*. — Journ. of Agric. Research, 60.
5. ÅKERBERG, E. 1942. Cytogenetic studies in *Poa pratensis* and its hybrid with *Poa alpina*. — Hereditas XXVIII.

ARNE MÜNTZING: Fertility improvement by recombination in autotetraploid *Galeopsis pubescens*.

It is a well known fact that experimental autotetraploids have a more or less reduced fertility in comparison with their corresponding diploids. In natural autopolyploids, on the other hand, fertility is much better. The causes underlying this difference between new and old autotetraploids were discussed in previous papers (MÜNTZING, 1936, pp. 313—322; 1937, pp. 216—217; 1939). It was concluded that the natural tetraploids have good fertility owing to natural selection and possibly also to secondary processes of various kind. It was also pointed out that the degree of sterility in experimental autotetraploids varies considerably in different species and biotypes, thus indicating that some species and biotypes are a more favourable material for natural selection than other ones. Finally, attention was called to the fact that the reduced fertility in experimental autotetraploids is not only caused by meiotic irregularities but is in the first place controlled by the genotypical constitution of the material.

To elucidate the problems in question experimental work is necessary. During the last years the present writer has obtained some data of this kind from work with various autopolyploids. At present, only some results gathered in *Galeopsis* will be reported, the material being two different autotetraploid strains of *G. pubescens* and their hybrid derivatives. The strains in question ($2n = \pm 32$) were produced by colchicine treatment of the diploid pure lines P—A and P—B ($2n = 16$), and some of their properties have already been described (MÜNTZING, 1941). In this abstract only the degree of seed setting will be considered. This property was measured in the diploid original lines, in the tetraploid strains and in the F_2, F_3 and F_4 generations following hybridization between the tetraploid strains.

The degree of female fertility is not easy to measure in *Galeopsis*, as the maximum number of seeds per calyx is four and each plant produces a very high number of flowers. However, a sufficient degree of accuracy was obtained by calculating the average number of seeds in ten open pollinated

TABLE 1. *Number of seeds per flower in diploid and tetraploid Galeopsis pubescens.*

Category	1940		1941		1942	
	n	M ± m	n	M ± m	n	M ± m
P—A, diploid pure line	43	1,69 ± 0,05	20	1,97 ± 0,10	20	2,13 ± 0,10
P—B, » » »	25	1,61 ± 0,08	16	2,04 ± 0,14	20	2,29 ± 0,08
P—A, tetraploid strain.....	48	0,30 ± 0,02	106	0,47 ± 0,02	98	0,36 ± 0,02
P—B, » »	27	0,43 ± 0,03	59	0,61 ± 0,04	74	0,31 ± 0,03
F_2—F_3, » , no selection ...	61	0,40 ± 0,02	21	0,59 ± 0,05	—	—
F_3—F_4, » , selection	—	—	92	0,67 ± 0,03	338	0,57 ± 0,02

flowers per plant. This was done in 1941 and 1942. In 1940 a higher number of flowers per plant were studied, but as the material increased in the following years the number of flowers examined per plant had to be reduced. — The data obtained are given in Table 1. The values in this table are based on a total of 1068 plants.

As is evident from the table seed setting in the diploid pure lines P—A and P—B does not reach the theoretical maximum of 4,00 seeds per flower, the average values ranging from 1,61 to 2,29. Evidently there is no difference in seed setting between the lines, the average values in each year being rather similar. On the other hand there are rather obvious differences between years, seed setting in 1940 being less good than in 1941 and 1942. Seed setting in the tetraploid P—A and P—B strains is very much reduced, the average values in the three years ranging from 0,30 to 0,61. If the diploid average seed setting (the average of both lines) is given the value 100, the corresponding figures of the tetraploid strains will be 22 in 1940, 27 in 1941 and 15 in 1942. Thus, considering all three years the seed setting in the autotetraploids is only about 21 per cent of that in the diploids. — The two tetraploid strains seem to differ in female fertility, the P—A strain having a significantly lower degree of seed

setting than tetraploid P—B in 1940 as well as 1941. In 1942, however, no such difference was obtained.

The facts of essential interest in Table 1 are represented by the values of seed setting obtained in F_2, F_3 and F_4 of the cross between the two tetraploid strains. In F_2, represented by 61 plants grown in 1940, the average number of seeds per flower was found to be $0{,}40 \pm 0{,}02$. This value is intermediate between the values obtained for the parent strains in this year, viz. $0{,}30 \pm 0{,}02$ and $0{,}43 \pm 0{,}03$. The F_3 generation grown in 1941 consisted of six progenies. Four of these represented a plus selection and were derived from the F_2 plants having the very best fertility, the number of seeds per flower ranging from 0,72 to 0,75. The average fertility in the offspring of these plants was found to be $0{,}67 \pm 0{,}03$. This value is higher than the average values of both parents grown in this year ($0{,}47 \pm 0{,}02$ and $0{,}61 \pm 0{,}04$). It is also somewhat higher than the average value ($0{,}59 \pm 0{,}05$) of two F_3 families derived from mother plants taken without selection. Thus, the data of the year 1941 indicate a positive result of the selection, but the differences are not yet great enough to be significant. However, after one more generation of selection a significant increase in fertility was really obtained. This is evident from the data of 1942. The F_4 generation grown in this year comprised 18 different progenies of mother plants which had an average seed setting ranging from 0,90 to 1,60. The average values of these 18 progenies ranged from 0,41 to 0,82 with a total average of the whole F_4 of $0{,}57 \pm 0{,}02$ seeds per flower (Table 1). In the same year the corresponding values of the parent strains were found to be $0{,}36 \pm 0{,}02$ and $0{,}31 \pm 0{,}03$ respectively. The differences in seed setting between the selected F_4 and the parent strains is rather great and quite significant. The total average of both parent strains will be 0,335 and the quotient $0{,}570 : 0{,}335$ is 1,701. Thus, seed setting in the selected F_4 was on an average 70 per cent higher than in the parent strains cultivated under the same environmental conditions.

There is every reason to believe that fertility in this material can be still more increased. Among the 18 F_4 progenies there was a significant heterogeneity, the average numbers ranging from $0{,}41 \pm 0{,}06$ to $0{,}82 \pm 0{,}06$. The latter value (based on 20 plants) represents an increase of 143 per cent in comparison with the total average of the parent strains, 0,335. This total average is based on 3 progenies of tetraploid P—A and 5 progenies of tetraploid P—B. The average values of the tetraploid P—A strains ranged from 0,35 to 0,38. The P—B progenies were more variable, the average values ranging from 0,17 to 0,42. The significant effect of the selection is very well illustrated by the fact that 17 of the 18 F_4 families had average values which were higher than any average in the parent strains. The increased seed production was also clearly visible by mere observations of the plants at the time of seed ripening.

So far even the best tetraploids have only a degree of seed setting corresponding to about 37 per cent of the seed setting in the diploid original lines. The important point, however, is the demonstration that *fertility in autotetraploids may be increased by recombination following hybridization.* This is of interest from theoretical as well as practical points of view. In maize which is cross-fertilizing RANDOLPH (1941) has already demonstrated that fertility in tetraploid strains could be improved by selection. Thus, there

is now positive evidence of this kind from allogamous as well as autogamous plant species. The evidence gathered in *Galeopsis* may be considered in more detail later on together with similar data obtained in tetraploid barley and rye. Also in material of ryewheat (*Triticale*) it is possible to increase fertility and yield by genetic recombination.

Literature cited.

1. MÜNTZING, A. 1936. The evolutionary significance of autopolyploidy. — Hereditas XXI, pp. 263—378.
2. — 1937. The effects of chromosomal variation in *Dactylis*. — Hereditas XXIII, pp. 113—235.
3. — 1939. Incompatibility and fertility in experimental and natural polyploids. — Proceed. of the 7th internat. Genetical congr., Edinburgh 1939, p. 224.
4. — 1941. New material and cross combinations in *Galeopsis* after colchicine-induced chromosome doubling. — Hereditas XXVII, pp. 193—201.
5. RANDOLPH, L. F. 1941. An evaluation of induced polyploidy as a method of breeding crop plants. — Am. Naturalist, Vol. LXXV, pp. 347—363.